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[54]发明名称 聚合物基质及其在药物组合物中的用途

[57]摘要

本发明提供了含有聚合物基质的药物组合物，特别是含有活性成分 IL-6 的组合物。也提供了特别的新的聚（碳酸亚乙酯）聚合物的更加完备的用途——在含药物活性化合物的缓释组合物中作为基质物质。提供了使用 IL-6 治疗 IL-1 和/或 TNFα 介导的疾病（特定的自身免疫或炎性疾病）和败血症休克的方法。

权 利 要 求 书

1. 一种可生物降解的聚合物，含有式A的碳酸亚乙酯单元:

－（－C（O）－O－CH_2－CH_2－O－）－　　A

其中碳酸亚乙酯的含量为70至100Mol％，在氯仿中在20℃测量特性粘度为0.4至4.0 dl/g，玻璃化温度为15至50℃。

2. 权利要求1的聚合物，以二氯甲烷为洗脱剂以聚苯乙烯为基准物通过凝胶渗透色谱检测其分子量（Mw）为100000至2000000.

3. 权利要求1的聚合物，其碳酸亚乙酯的含量为90－100 Mol%。

4. 权利要求1的聚合物，其比浓对数粘度在浓度为1 g/dl的氯仿中测定为0.4-3.0 dl/g。

5. 权利要求1的聚合物，其玻璃化温度为18至50℃。

6. 权利要求1的聚合物，其含有碳酸亚乙酯单元和环氧乙烷单元。

7. 权利要求1的聚合物，其在生理条件下通过水解酶或通过pH 12、37℃的水在至少1个月内不明显降解。

8. 权利要求7的聚合物，接触沸腾的双蒸水5小时后其玻璃化温度为18至50℃.

9. 权利要求1的聚合物，在过氧自由基阴离子O_2^-影响下在体内和体外通过表面溶蚀降解。

10. 权利要求9的聚合物，它表现为连续的物质降解而残余物的分子量不降低。

11. 权利要求10的聚合物，它在5天至6个月内被生物降解。

12. 权利要求1－11任意一项的聚合物，其具有式B的环氧乙烷氧单元:

$-(-CH_2-CH_2-O-)-$ B

作为共聚单元。

13. 权利要求1－12任意一项的聚合物，它含有羟基作为聚合物的一个末端基团。

14. 权利要求1－12任意一项的聚合物，它含有酯基作为聚合物的一个末端基团。

15. 权利要求14的聚合物，其表面溶蚀速率通过选择末端酯基调节。

16. 制备权利要求1－12任意一项的聚合物的方法，其中环氧乙烷和二氧化碳是以1：4至1：5的摩尔比在催化剂的作用下聚合。

17. 权利要求16的方法，其中所用催化剂由二乙基锌和水或丙酮以0.9：1至1：0.9的摩尔比制备。

18. 权利要求16的方法，其中所用催化剂由二乙基锌和二－或三酚以2：1至1：2的摩尔比制备。

19. 权利要求16的方法，其中所用催化剂由二乙基锌和二醇以0.9：1至1：0.9的摩尔比制备。

20. 权利要求19的方法，其中所用催化剂由二乙基锌和1，2－亚乙基二醇制备。

21. 权利要求18的方法，其中所用催化剂由二乙基锌和间苯三酚制备。

22. 权利要求16至21任意一项的方法，它是溶剂或在有机溶剂和二氧化碳的分散试剂系统中进行的。

23. 权利要求16－22任意一项的方法，它是在压力为20至70巴温度为10至80℃的条件下进行的。

24. 制备具有权利要求13的羟基作为（共）聚合物末端基团的聚合物的方法，它是按照权利要求17至22任意一项的方法进行的。

25. 制备具有权利要求14的酯基作为聚合物末端基团的聚合物的方法，它是按照权利要求17至22任意一项的方法进行并用任意的酯化反应完成。

26. 含有药物活性化合物的药物组合物，其中药物活性化合物存在于非水解表面溶蚀的聚合物中。

27. 权利要求26的存在于聚合物中的药物活性化合物的药物组合物，其中活性化合物的释放与非水解聚合物物质降解呈直线关系并且活性化合物保护于聚合物基质中。

28. 一种在权利要求1－15任意一项的聚合物中的药物组合物。

29. 权利要求26或27的药物组合物，其中含有活性蛋白质或肽。

30. 权利要求29的药物组合物，其中含有细胞因子。

31. 权利要求30的药物组合物，其中含有白细胞介素。

32. 权利要求26的药物组合物，其特征是以微粒或植入剂的形式存在。

33. 权利要求26－29任意一项的药物组合物，在聚合物中或上含有添加剂。

34. 权利要求33的药物组合物，其中含有自由基清除剂作为添加剂。

35. 权利要求33的药物组合物，其中含有多元醇作为添加剂。

36. 权利要求35的药物组合物，其中含有糖醇作为添加剂。

37. 权利要求36的药物组合物，其中含有甘露糖作为添加剂。

38. 权利要求33－35任意一项的药物组合物，其中含有占总重量1至90％的添加剂.

39. 权利要求26的药物组合物用于白细胞介素或CSF的非肠道给药.

40. 权利要求39的药物组合物，其中白细胞介素或CSF存在于如权利要求1限定的式A聚合物中.

41. 权利要求39的组合物的用药方法，其中包括给需此治疗的患者通过非肠道给药.

42. 权利要求26－40任意一项的药物组合物，其中包含活性成分IL－6.

43. 一种其中于聚合物基质中含IL－6的药物组合物.

44. 权利要求43的药物组合物的微粒或植入剂剂型.

45. 权利要求44的药物组合物用于治疗自身免疫或炎性疾病.

46. 权利要求45的药物组合物，其中所治疗的疾病是多发性硬化.

47. 权利要求45的药物组合物，其中所治疗的疾病是类风湿性关节炎.

48. 权利要求45的药物组合物，其中所治疗的疾病是莱姆病.

49. IL－6在制备下调或抑制IL－I和/或TNFα药物中的用途.

50. 权利要求49的IL－6在制备治疗慢性或急性病原体诱发的炎性疾病或脱髓鞘疾病的药物中的用途.

51. 权利要求50的用途，其中所治疗的是败血症休克.

52. 权利要求50的用途，其中所治疗的是多发性硬化.

53. 权利要求50的IL－6的用途，其中所治疗的是莱姆病.

54. 权利要求49－53任意一项的用途，其中IL－6是重组人IL－6.

说 明 书

聚合物基质及其在药物组合物中的用途

本发明涉及含有聚合物基质、特别是含有用于治疗 IL - 1 和/或 THF α介导的疾病（例如慢性炎性疾病）的 IL - 6 的药物组合物。在此描述的本发明特殊聚合物（特别是聚（碳酸亚乙酯）聚合物），在含有药用活性化合物的缓释组合物中一般将它用作基质物质，特别是它在体内进行非水解表面溶蚀的性质是新的、出乎意料的，且特别符合要求。因此，含有其它药物的基质及聚合物的制备方法和含此聚合物的药物组合物也在此举例说明。此外， IL - 6 治疗 IL - 1 和/或 TNF α介导的疾病的用途是新的并出乎意料（以前认为很多这样的疾病会因 IL - 6 而恶化），于是本发明进一步提供了 IL - 6 在治疗如慢性病原体诱发的炎性疾病、脱髓鞘疾病及急性和超急性炎性疾病（如败血症休克）中的新用途。

I. 治疗 IL - 2 和/或 TNF α介导的疾病

很多自发的慢性炎性疾病病因不明（可能是自身免疫）并被认为是由 IL - 1 和/或 TNF α介导的。例如，多发性硬化（ MS ）——一种特征为在脑和脊髓中存在脱髓鞘的播散斑的残缺神经紊乱，在很多年里成为研究单位的注意焦点。虽然多发性硬化的准确病因并不完全清楚，但根据征兆（如疾病患者的某种 HLA 抗原的频率增加）人们相信它存在强自身免疫因素。通常提供的抗炎药如 ACTH（促肾上腺皮质激素）或皮质甾类（如强的松）似乎会促进急性病的康复（特别是在早期使用），但不能治本。长期使用皮质甾类或免疫抑制剂会有严重的副作用。最近表明 IFN - β 1 合剂降低短期的斑形成，但没有长期疗效。治疗效果的评估

很复杂，因事实上疾病的自然进程会自发地缓解并慢性复发．简言之，尽管广泛研究了很多年，但对这种严重的疾病还没有总体上可接受的特别治疗方法．

其它慢性炎性疾病被认为是由外源试剂（如病原体）诱导的．例如，莱姆病是由蜱生螺旋体疏螺旋体属 burgdorferi 感染引发的严重慢性疾病．起初急性期症状为皮肤损伤及流感样症状，之后疾病进入慢性期，它的症状为关节炎及慢性神经异常．通常用抗菌素和非甾类抗炎药治疗此疾病，但特别是对此疾病患者尚未发现适当的治疗方法．

急性或超急性不可控制的炎性疾病也可由内源性试剂引发（如严重的烧伤或严重的感染）．因目前尚无有效治疗方法，败血症休克是严重威胁生命的疾病．成人呼吸窘迫综合征（ ARDS ）也尤为如此．它的发病快，死亡率一般超过 50 %．败血症休克通常是严重细菌感染的结果，它的典型症状是发烧后期常伴有低温、血压波动（肌力过度症状）后期伴有低血压、代谢酸中毒、智力损伤及广泛的器官功能障碍，在很多病例中最后导致死亡．最常见的败血症休克是因革兰氏阴性细菌感染（内毒素），但也可因革兰氏阳性感染或其它感染．于是将在此使用的术语“败血症休克”解释成广义的由微生物感染（特别是细菌感染，最特别是革兰氏阴性细菌感染）引起的休克状态（包括 ARDS ）．

IL-6 是已知的细胞因子．可用于多种疾病的治疗，例如，血小板减少症及特定的癌症．它通常随机体对细菌感染的免疫而产生并且和炎症、发烧及败血症休克的调节有关．它是强免疫刺激剂，确有一些文献指出 IL － 6 作用机制引起某种自身免疫或炎性疾病，包括系统性红斑狼疮、多发性硬化、类风湿性关节炎及败血症休克．

因此，人们非常惊奇地发现 IL － 6 可用于治疗慢性炎性疾病（除肾

小球性肾炎外），如多发性硬化，及用于治疗急性和超急性炎性疾病，如败血症休克．其作用机理尚不清楚，但我们相信不用考虑任何特别的理论，只是通过反馈机理， IL － 6 可降低或抑制其它细胞因子的表达、释放或功能，特别是对 TNF α和/或 IL － 1 ，可能通过上升调节可溶性 TNF α受体和/或 IL － I 受体拮抗剂的释放，抑制其活性及其主要是这些细胞因子介导产生的自身免疫、炎症或休克症状．但在特征为 IL － 6 介导的补体激活抗原－抗体（ IgG ）复合物的疾病中，特别是肾小球性肾炎（它通常由这样的复合物在肾中沉积引起）， IL － 6 会加重病情．因此，我们强调 IL － 6 对动物模型的 MS 和莱姆氏关节炎（主要由 IL － I 和/或 TNF α造成）有治疗作用，但会加重狼疮鼠的肾小球性肾炎（直接由 IL － 6 造成）．我们也指明 IL － 6 自身对内毒素休克（同样假设其主要由 IL － I 和/或 TNF α造成）的鼠模型有治疗作用．

因此，认为特别是在治疗除肾小球性肾炎外的炎性疾病及治疗败血症休克中 IL － 6 可用作抑制 TNF α和/或 IL － I 的表达、释放或功能的试剂．可以用 IL － 6 治疗的炎性疾病包括如关节炎，特别是病原体诱发的关节炎如莱姆病关节炎、细菌诱发的关节炎及脊髓灰质炎性关节炎；多发性硬化及其他脱髓鞘疾病（即神经、大脑和/或脊髓脱髓鞘为特征的疾病，包括如多发性硬化、急性散布性脑脊髓炎或传染病后脑炎、眼神经脊髓炎、耳鸣、弥散性大脑硬化、席尔德病、肾上腺白质营养不良、第三期莱姆病、 tropical spastic parapoesis,及脱髓鞘特别是自身免疫介导的脱髓鞘为主要症状的其它疾病）；急性重症炎性疾病如烧伤、败血症休克、脑膜炎及肺炎；以及自身免疫疾病包括多软骨炎、硬皮病、韦格纳 granulamatosis 、皮肤肌炎、慢性活动性肝炎、重症肌无力、牛皮癣关节炎、史蒂文斯－约翰逊症、特发性口原性腹泻、自身免疫性

肠炎（包括如溃疡性结肠炎和节段性回肠炎）、内分泌眼病、凸眼性甲状腺肿、肉样瘤病、原发性胆汁型肝硬变、青少年糖尿病（Ⅰ型糖尿病）、眼色素层炎（前和后）、干性角膜结膜炎和春季角膜结膜炎以及间质性肺纤维化。

因此本发明提供了

i)抑制 TNF α和/或 IL - Ⅰ的表达、释放或功能的方法；

治疗或预防除肾小球肾炎外的炎性疾病的方法；

治疗或预防 TNF α和/或 IL - Ⅰ介导的疾病的方法；

治疗或预防上述任何疾病的方法；

治疗或预防脱髓鞘疾病如多发性硬化的方法；

治疗或预防外部诱发的炎性疾病的方法；

治疗或预防重症急性感染如败血症休克脑膜炎或肺炎的炎性反应；

治疗烧伤的方法；

治疗或预防病原体诱发的慢性炎性疾病如莱姆病的方法；

所述方法包括使用治疗或预防有效剂量的 IL - 6，例如 TNF α和/或 IL - Ⅰ抑制量的 IL - 6 如 rhIL - 6（如特别是当 IL - 6 作为单一的治疗或预防试剂使用时，或者与抗微生物或作用于血管的药剂任意联合使用，如任选不与 TNF α激动剂或拮抗剂或抗 TNF α抗体联合使用）；任选以缓释或贮存剂型如与聚合物基质（如下文描述的聚（碳酸亚乙酯）基质）联合用于需此治疗或预防的对象如哺乳动物、人；

ii)在制备用于方法（ i ）如治疗或预防列于上述（ i ）的任何一种疾病的药物中 IL - 6 如 rhIL-6 的用途，其中药物是任意的缓释制剂如任选进一步含有聚合物基质（如下文描述的聚（碳酸亚乙酯）基质）；

iii)治疗或预防列于上述（ i ）中任何疾病中 IL - 6 如 rhIL - 6 的用途；及

iv)含有 IL - 6 如 rhIL - 6 的药物组合物，将其用于（ i)方法中，如治疗或预防上述（ i ）描述的任何疾病，任选以缓释制剂的形式，任选进一步含有聚合物基质（如下文描述的聚（碳酸亚乙酯）基质）；例如，在聚合物基质中含有 IL - 6 的缓释组合物（即在几天、几星期或几个月的时期内在体内生物降解的组合物），如以微粒或贮库的形式，其中聚合物在体内显示为非水解溶蚀，特别是本文描述的任何给药系统用于治疗上述任何疾病（如慢性炎性疾病）时。

IL - 6 指相应与白细胞介素 - 6（也称为 β - 2 干扰素（ INF - β II ）、B 细胞因子 - 2 （ BSF - 2 ）、白细胞介素 - HP - 1 （ HR1 ）、肝细胞激活因子（ HSF ）、杂交瘤浆细胞瘤生长因子（ HPGF ）及 26kD 因子）的已知变体的任何化合物. 虽然非重组 IL - 6 也可使用，但优选重组 IL - 6（如由 IL - 6 分泌癌细胞系产生的）. IL - 6 可以购买或通过任何已知的方法制备（如 EPA0220574 、 EPA0257406 、 EPA0326120 、 WO88/00206 、 GB2063882 或 GB2217327 描述的，这些申请的内容在此引为参考）. 可将 IL - 6 糖基化（如由真核细胞如 CHO 细胞产生的）或非糖基化（如由原核细胞如 E ， Coli. 产生）. 虽然已知 IL - 6 为活性杂交种，但优选重组人 IL - 6 （ rhIL - 6 ），也可使用来自非人源的 IL - 6 并将其包括在本文 IL - 6 的含义范围内. 在 IL - 6 的序列中具有微小变化（如增加、删除或诱变 1 、 2 、 3 或多个氨基酸）的蛋白质、含有 IL - 6 及其它蛋白的融合蛋白质、 IL - 6 的活性片断和/或其它具有 IL - 6 活性的 IL - 6 的变体、截断的或突变的形式包括在本文 IL - 6 的含义范围内.

含有IL－6和药用稀释剂或载体的适宜的药用组合物是已知的。IL－6可非肠道给药，如以注射溶液或混悬液的形式，按照或类似于Remington's Pharmaceutical Science,第16版（Mack Publishing Company,Easton,PA 1980）的描述。适宜的载体包括水载体如生理盐水、Ringer氏溶液、右旋糖溶液及Hank氏溶液以及非水载体如脂肪油和油酸乙酯。对于一般的非肠道给药，单位剂量的IL－6以冻干的形式，它可与载体混合形成适当的注射用溶液或混悬液。

另外，IL－6可用植入或缓释药物给药系统，如微粒或贮库制剂与聚合物一起形成聚合物基质，而药物从基质中缓慢释放。在所治疗的疾病为慢性病（如慢性炎性疾病）且所需治疗持续若干星期或若干月时优选这种方式。聚合物是指由重复单元任何适当（如可药用）线性高分子量分子（包括均聚物、共聚物及杂聚物）组成，其可是任意的支链或胶联的形式，如通过一种分子聚合或多于一种分子共聚（如由下文描述的环氧乙烷和二氧化碳形成的聚（碳酸亚乙酯）），并且可在聚合物链上任意含有其它单元的嵌段。优选的聚合物为线性且由碳、氧和氢组成，如聚－DL－丙交酯－乙交酯共聚物、聚乙二醇或聚（碳酸亚乙酯）。优选的聚合物表现出非水解溶蚀，如本文进一步描述的聚（碳酸亚乙酯）。

所用的剂量当然随所用IL－6的准确类型、宿主、给药方式及治疗病症的特点和严重程度变化。通过皮下注射或缓释剂型给较大的哺乳动物用药的每日剂量为0.5μg/kg至30μg/kg，优选2.5μg/kg至10μg/kg，或以IL－6的其它任何安全及有效的体内活性剂量用于治疗，如以血小板增加的剂量。在重症急性炎性疾病如败血症休克中，需要较高剂量静脉给药以达到快速和较强的反应。IL－6给药的频率可由每日给药降至隔日或每星期或在使用缓释剂型时间隔更长的时间给药（这在

长期治疗时优选）。 IL - 6 治疗可导致寒战、发烧及流感症状，这一般可共同使用非麻醉止痛药如阿斯匹林、扑热息痛或消炎痛治疗或预防。其它显著的副作用一般仅在高剂量如高于每日 10 μ g/kg 时出现，并一般可通过降低剂量缓解。

II.缓释用聚合物基质

本发明进一步提供适于药物缓释的药物组合物，它适于在上述征兆时 IL - 6 及其它药物的给药。此药物组合物特指那些含有聚（碳酸亚乙酯）聚合物，有时称作聚（碳酸亚乙酯）类或 PEC 类。

虽然现有技术提供了一些聚（碳酸亚乙酯）类用于药物给药系统的实例，但现有技术并未公开本发明的特定聚合物，同时也未公开此聚合物能在体内进行非水解溶蚀。现有技术也未公开本文公开的特定药物（如 IL - 6 ）的给药系统，也未提出这样的药物需缓释系统。

特别令人惊奇的是本发明聚合物的生物降解特性。根据一般化学基础知识，预计碳酸酯键为主要的裂解键。然而，聚碳酸酯在体外适当的条件下是稳定的。

按照 Chem. Pharm. Bull. 31(4),1400-1403(1983)，聚（碳酸亚乙酯）类可在体内进行生物降解，但所试的聚合物不能通过如现代光谱法明确地鉴定。按照 1402 页，体内生物降解只能解释为由于水解酶的作用。

按照 Chem. Pharm. Bull. 32(7),2795-2802(1984)，由含地布卡因的聚（碳酸亚乙酯）制成微粒。虽然该描述涉及非常相关的技术，但从中并未看出地布卡因的释放与聚合物的体外或体内生物降解有关。同样所试聚（碳酸亚乙酯）的物理及化学性质未得到充分说明。

按照 Makromol. Chem. 183, 2085-2092(1982)（特别是 2086 页），

二氧化碳环氧乙烷聚合物可生物降解，它还指出初步的结果证实了二氧化碳－环氧乙烷聚合物的生物降解性及其由此带来的它们在控制药物释放中的应用。为支持生物降解性的断言它引用了 Jinko Zoki 3(Suppl.),212(1974)。此出版物说明聚（碳酸亚乙酯）属最易水解的一组化合物，甚至链霉蛋白酶在分解它时也毫无困难。既然链霉蛋白酶由水解酶混合物组成，这意味着在体外及体内酶水解是可能的。但这个结论非常令人怀疑。我们将本发明的聚（碳酸亚乙酯）类以直径 5mm 、重量 25mg 的压片形式加入到 10mg/ml 链霉蛋白酶和 5mM $CaCl_2 \cdot 2H_2O$ 的 pH 7.4 的磷酸盐缓冲生理盐水（ PBS)液中及 10mg/ml 链霉蛋白酶 E 和 5mM $CaCl_2 \cdot 2H_2O$ 的 pH 7.4 的磷酸盐缓冲生理盐水（ PBS ）液中（在 37 ℃）没有观察到生物降解（见图 1 ）。链霉蛋白酶溶液每天更新。

现在惊奇地发现选择具有特殊碳酸亚乙酯的含量、粘度及玻璃化温度范围的不能水解生物降解（如在水解酶如链霉蛋白酶的存在下或在碱性条件下）的聚（碳酸亚乙酯）类在体外和体内不再生物降解，即只是通过表面溶蚀。“表面溶蚀”的表达方式用于本发明，特别与聚酸酐及聚原酸酯的水解生物降解有关，但决不仅仅限定于此。

如果物质的生物降解仅仅在聚合物颗粒的表面，而其余聚合物残留物的分子没有降低，表面溶蚀便发生了。在文献中声明观察到的表面溶蚀中，没有进行符合物质损失检测的残余物分子量测定，因此实际上从未证实过表面溶蚀。

实际上，对至今几乎所有所试的聚合物，只观察到聚合物整体溶蚀。具有聚合物整体溶蚀的系统有显著的缺点，即如果聚合物载有药物化合物如肽，肽可能释放到生物介质中，在生物介质的影响下相当不稳定。药物化合物的主体部分已与介质接触，早在其从聚合物中释放前便可能

失去活性。如果聚合物能进行表面溶蚀，即当没有整体溶蚀发生时，植入的药物化合物（如肽）可在表面溶蚀达到药物颗粒及药物颗粒从残余聚合物的表面释放前得到保护，不受生物介质的有害影响。在聚合物基质药物系统中，显示有表面溶蚀而不是整体溶蚀，药物颗粒接触生物介质的有害影响的时间较短，由此能使药物活性物质从聚合物基质中的释放时间较长、量较高并且较稳定。

在最近的出版物 Proc. Nat. Acad. Sci. USA 90552-556(1993) 和 904176 - 4180 (1993)中描述了对聚酸酐的类似表面溶蚀的一些特性。但是仍受整体溶蚀的影响并且没有进行分子量测定。此外这种溶蚀是水解类型的。现在发现选择聚（碳酸亚乙酯）类的组份（在下文定义）表明在体外及体内绝对都是非水解表面溶蚀。

本发明提供了在体内及体外通过由非水解机理控制的表面溶蚀降解的聚合物，它具有式 A 的碳酸亚乙酯单元:

$$-(-C(O)-O-CH_2-CH_2-O-)- \qquad A$$

其中碳酸亚乙酯的含量为 70 至 100 Mol %，在氯仿中在 20 ℃测量特性粘度为 0.4 至 4.0 dl/g，玻璃化温度为 15 至 50 ℃。

本发明的聚合物的碳酸亚乙酯的含量为 70 至 100Mol %，特别是 80 - 100 %，优选 90-99.9%，如 94-99.9%。聚合物的特性粘度在氯仿中在 20 ℃测量为 0.4 至 4.0 dl/g。优选聚合物具有比浓对数粘度（在 20 ℃及粘度为 1g/dl 的氯仿中测量）为 0.4 至 3.0 dl/g。

玻璃化温度为 15 至 50 ℃，优选 18 至 50 ℃。

在文献中已描述了玻璃化温度为 5 至 17 ℃的聚（碳酸亚乙酯）类.

本发明的聚合物优选由环氧乙烷和二氧化碳共聚制备，其制备方法也是本发明的一部分。作为本制备方法的结果，在大多数实例中聚合物

含有式 B 的环氧乙烷单元作为共聚单元

$-(-CH_2-CH_2-O-)-$ B

如果本发明的聚合物接触水介质（如 pH7.4 磷酸盐水缓冲液），实践证明没有介质迁移到它们的主体部分（见图 2）。因此在至少 28 天内未发生整体溶蚀并且残余物保持常数（100 %），见图 3 中的右图。

目前聚－ DL －乙交酯－丙交酯共聚物是最常用的缓释药物系统的基质物质。但这样的聚合物与本发明的聚合物不同，通过水解降解。例如，在 PBS 中的物质降解显示于图 3 的左图，这是最复杂的聚－ DL －乙交酯－丙交酯共聚物类型之一，即葡萄糖引发的聚－ DL －乙交酯－丙交酯共聚物（DL － PLGGLU），描述于英国专利 GB2145422。

本发明的聚（碳酸亚乙酯）类与现有技术的聚－ DL －乙交酯－丙交酯共聚物之间的体内降解行为的不同也如图 3 所示。其中聚乙交酯－丙交酯共聚物进行整体溶蚀，正如所见的 DL － PLGGLU 的残余物的分子量降低，而聚（碳酸亚乙酯）类的残余物的分子量保持常数（100 %）。

在一个月内两个例子中体内的总植入物的残余物降至零，它意味着聚（碳酸亚乙酯）进行表面溶蚀而不是整体溶蚀。作为缺乏整体溶蚀的结果，在贮存期间（即在给药前）载体聚合物不让潮气渗入，保持与制备时相同的干燥状态。如果其中包藏的药物对潮湿敏感则也会保持稳定。

本发明也提供了制备聚合物的方法，其中环氧乙烷和二氧化碳以 1： 4 至 1： 5 的摩尔比在催化剂的作用下聚合。如果两个环氧化合物的分子互相反应而没有二氧化碳分子的干扰，即如果含氧阴离子中间体在被二氧化碳羧基化前进攻另一个环氧乙烷分子，则很清楚在本反应的范围内在聚合物链上引入环氧乙烷单元是可能的。这样聚合物可能含有

若干个环氧乙烷单元。本发明的聚合物如果含有环氧乙烷单元则具有碳酸亚乙酯和环氧乙烷单元的随机分布，表示为总式 Am-Bn=

$$-(C(O)-O-CH_2-CH_2-O-)-m-(-CH_2-CH_2-O-)-n$$

其中

$$\frac{m}{n+m} \times 100 = 70 \text{ 至 } 100$$

但是本发明聚合物中的多数环氧乙烷单元据统计具有邻近的碳酸亚乙酯单元，特别是在环氧乙烷单元的摩尔比例小的实例中。这意味着在这些实例中所得的多数醚官能团随机分布在聚合物链的碳酸官能团之间。在 CDC13 中本发明产品的 1H - NMR 证明了这种假设。它们在 δ = 约 4.37ppm(全部 Ia ）显示了碳酸亚乙酯单元（在两个碳酸官能团之间的乙烯单元）、在约 4.29 和 3.73ppm （全部 Ib 和 Ic ）在一个碳酸和一个醚官能团之间的乙烯单元及在约 3.65ppm （全部 Id ）在两个醚官能团之间的乙烯单元的信号。碳酸亚乙酯单元的比例（ A ）就可在 NMR 精确度内按下式计算:

碳酸亚乙酯单元的 Mol%（ A ）：

$$\frac{Ia}{Ia+Ib+Ic+Id} \cdot 100$$

作为聚（碳酸亚乙酯）类的结构特征，在文献中常给出它们的醚官能团的含量，而不是其碳酸亚乙酯的含量。本发明的聚合物中醚官能团的比例（ E ）可按下式计算:

醚官能团的 Mol%(E)=

$$\frac{Ic+Id}{Ia+Ib+Ic+Id} \cdot 100$$

按照 PCT 专利申请 WO92/22600 制备聚（碳酸亚乙酯）类，其中以 2 至 400 : 2 的摩尔比含环氧乙烷单元和碳酸亚乙酯单元，这意味着聚合物含有至少 50Mol %的环氧乙烷和少于 50Mol %的碳酸亚乙酯单元。此申

请提到聚合物的生物降解性和它们作为生物可溶蚀基质用于缓释药物活性化合物的用途。但是没有给出数据证实聚合物确实能生物降解。总之，具有如此大量的醚官能团的聚（碳酸亚乙酯）类很少能生物降解。此申请未提及任何有关此聚合物表面溶蚀可能性的暗示。

美国专利 3248415 的实施例中描述了含有少于 70Mol %的碳酸亚乙酯单元的低分子量聚（碳酸亚乙酯）类（ Mw = 700 - 5000 ），这不同于本发明的聚合物并且未提及任何有关的生物降解性。

PCT 专利申请 WO89/05664 描述的聚（碳酸亚乙酯）类含有结构 II 环氧乙烷和碳酸亚乙酯单元的摩尔比为 1 至 8： 1，这意味着聚合物含有至少 50Mol %的环氧乙烷和最多 50Mol %的碳酸亚乙酯单元，这不同于本发明的聚合物。虽然描述了此聚合物用于可生物降解的给药器如含有药物化合物的植入物，但没有给出有关表面溶蚀的信息。

本发明的方法中，环氧乙烷单元的含量和醚官能团的含量延缓或抑制了聚合物的生物降解速度，通过选择反应条件如反应组份的摩尔比率、反应温度并进一步选择合适的催化剂降低其含量，催化剂可由二乙基锌和水或丙酮或二－或三酚（如间苯三酚）分别以摩尔比 0.9:1 至 1:0.9 或 2： 1 至 1： 2 制备，或优选由二乙基锌和二醇(特别是乙二醇)以 0.9:1 至 1:0.9 的摩尔比制备。

此方法优选在有机溶剂（如二□恶烷和二氧化碳）的溶剂或分散剂系统进行。优选二氧化碳以液体形式使用并过量存在。压力优选 20 至 70 巴并优选温度为 10 至 80 ℃，特别是 20 至 70 ℃。

这样制得的本发明聚合物通常含有少于 15 %的醚官能团，优选少于 10 %，特别是少于 5 %（如少于 3 %）。本发明的聚（碳酸亚乙酯）类如果使用由甘醇或丙酮和二乙基锌制备的催化剂制备则其表现有低多分

散性（ Mw/Mn ），通常小于 5 ，如小于 2.5 。

本发明的方法中催化剂或部分催化剂被看作是（共）聚合物的链引发剂。当反应完成且链生成时，其末端基团是羟基。链的相反位点（链的起始点）可被催化剂基团或其片段占据。如果催化剂由乙二醇和二乙基锌或水和二乙基锌制备，则聚合物链的两端相同。但是如果催化剂是由二－或三酚和二乙基锌制备的，芳族基团将被引入链的末端（这是链的起始点），而链的另一端为羟基。由图 4 可见，如果聚（碳酸亚乙酯）的一个末端基团被封端，例如，被如间苯三酚的芳香引发剂封端，则它的生物降解较慢。因此人们假设聚合物链降解起始于一个羟基末端或多个羟基末端。或者，也可考虑末端羟基的随后衍生化（如通过酯化）封端羟基并控制本发明的聚（碳酸亚乙酯）类的生物降解。适宜的末端酯基团为生物相合的酯基团，如（ C_{1-48} ）脂肪酸酯基团，优选（ C_{1-30} ）特别是（ C_{1-18} ）的脂肪酸酯基团，例如乙酸和硬脂酸的基团，或碳酸酯基团如碳酸亚乙酯基团、或双羟萘酸酯基团或乳酸、或乙醇酸、或聚乳酸、或聚乙醇酸、或聚乳酸－共－乙酸酯基团。

本发明的聚（碳酸亚乙酯）类在热水（ 90 － 100 ℃）中可稳定数小时，而没有明显的分子量降低。在接触沸腾的双蒸水 5 小时后观察到玻璃化温度显著增加，如达到高于 18 ℃（如 28 ℃）。通过这个反应步骤，得到较高的聚合物纯度。我们发现用此方式处理聚合物也较容易。

如前所述，本发明聚合物的聚（碳酸亚乙酯）部分是不可水解的，即在生理条件下通过水解酶或在 pH12 及 37 ℃至少一个月内不水解（见图 1 和 8 ）。但是发现本发明的聚合物在体内和体外在过氧自由基阴离子 O_2^- 的作用下通过表面溶蚀降解。过氧自由基阴离子 O_2^- 在体内和体外的炎性细胞中在本发明聚（碳酸亚乙酯）类存在下产生（见图 5 ）。聚

乙交酯－丙交酯共聚物目前最常用作药物缓释系统的基质物质，通过整体水解降解，不能诱发过氧自由基阴离子O_2^-的产生，在同一图中显示了葡萄糖引发的聚－DL－乙交酯－丙交酯共聚物，也可见图3。

在体外建立了含有过氧化钾的水溶液体系作为O_2^-的来源，并显示了本发明的聚（碳酸亚乙酯）类的表面溶蚀（见图8）。在体外选择pH12，因为O_2^-自由基在此pH值充分稳定。

有趣的是，不同于乙烯单元的氢被甲基取代的聚（碳酸亚乙酯），聚（碳酸亚乙酯）几乎不可生物降解，见 Chem. Pharm. Bull 31(4),1400-1403(1983)。

使用本发明的聚（碳酸亚乙酯）类的微粒混悬液在48只鼠和24只狗分别进行了21天和35天的毒理研究。每种在第1天和第17天使用两次。经皮下及肌肉使用聚合物微粒10和40mg/kg体重后，未观察到系统临床毒性，未观察到对血液学参数、临床血液化学参数、体重和进食量的相关影响。在给鼠用药4和21天及给狗用药18和35天后测定用药位置的组织病理学变化。除了预期的炎性反应外，未发现不正常的组织病理学变化。

本发明聚合物的降解速率可在大范围内调整，这依靠于它们的分子量、环氧乙烷的含量、末端基团的性质（如生物相合的酯基团）、及O_2^-自由基清除剂（如维生素C）的存在，并可持续5天至6个月或更长，如高达1年。自由基清除剂优选作为添加剂置于聚合物中。

本发明的（共）聚合物的分子量Mw为80000（优选100000，特别优选200000）至2000000道尔顿，这可用二氯甲烷作为洗脱剂以聚苯乙烯作为参照物通过凝胶渗透色谱测定。

上面讨论的 Chem. Pharm. Bull. 32(7) 2795-2802(1984)提到使用

了分子量为 50000 至 150000 道尔顿的聚（碳酸亚乙酯）类。我们发现聚合物在体内和体外降解只有在分子量高于 80000（优选 100000）（图 6）时才能令人满意。这也正是本发明的内容。

本发明的聚合物可用于药物组合物，特别是作为包藏药物活性化合物的基质物质。因为在体外和体内条件下未发生整体溶蚀并且活性化合物被聚合物保护，故由于基质的表面溶蚀活性化合物一出现在基质表面（而不是在这之前）就立即释放。在体外 pH7.4 不含 O_2^- 的水溶液系统中，只有痕量的活性化合物释放（见图 9）。

表面溶蚀的另外一个优点是药物活性化合物分子的大小不影响释放的速率。

于是本发明提供了一种存在于聚合物中的药物活性化合物的药物组合物，此聚合物表现出非水解表面溶蚀，特别的活性化合物的释放与非水解聚合物的物质降解是直线关系（特别是 1： 1 的直线关系）且活性化合物在聚合物基质中得到保护。

组合物优选以微粒或植入片的形式使用。

可通过已知的方法制备本发明药物的制剂，通过适当的喷雾干燥或乳化技术制备微粒，通过将药物化合物和聚（碳酸亚乙酯）类的固体颗粒混合，在高温下聚（碳酸亚乙酯）类软化而便于处理，随后任意将混合物冷却成固体并制成适宜的形状。也可将溶解或分散状态的药物化合物与聚（碳酸亚乙酯）溶液混合并蒸发溶剂，此后将固体残余物制成适当的植入片剂型。

含有微粒的药物组合物可通过将其与适当的草本制剂赋形剂加工处理并将其任意置于适当的分散剂中制备。

根据药物性质及制备方法，药物的含量可在大范围内变化，其重量

比由 0.001 至约 70 %，如 0.001 至 20 %，优选 0.001 至 5 %。应避免药物含量高导致介质渗透入聚合物中，这限制了加药量的上限。

在使用药物化合物的医学实践中，药物活性化合物的每种类型都可用于与本发明的聚（碳酸亚乙酯）合用。对于微粒优选的药物化合物类型是在低含量具有药物活性并在长时期内其血药浓度需恒定，如激素、肽或蛋白质（如生长激素释放抑制因子、干扰素或细胞介素），但特别优选那些不稳定并且口服后在胃肠系统会分解并因此非肠道给药的药物。

本发明的贮库剂型可用于多类活性制剂的给药，药物活性制剂如避孕药、镇静药、甾类、磺胺类、疫苗、维生素、抗周期性偏头药、酶、支气管扩张剂、心血管药物、止痛药、抗菌素、抗原、抗痉挛药、抗炎药、抗帕金森药、催乳激素抑制剂、抗哮喘药、老年病用药及抗肿瘤药。活性制剂可在广泛的化学化合物中挑选，如亲脂性或亲水性活性制剂，包括肽如 octreotide（描述于英国专利 GB 2234896 A）。

活性蛋白质或肽优选细胞因子，如白细胞介素、G - CSF、M - CSF、GM - CSF 或 LIF、干扰素、红细胞生成素、环孢菌素或激素，或它们的类似物（如 octretide）。

药物组合物可用于:

免疫调节,其中活性成分包括细胞因子如白细胞介素（IL - 3、IL - 6）或成血集落刺激因子（G - CSF 如 Filgrastim、GM - CSF 如 Molgramostim、Sargramostim、M - CSF），如作为疫苗的辅剂;

在骨髓抑制治疗或骨髓移植后重新造血的目的，活性成分包括成血生长因子如 GM - CSF、G - CSF、IL - 3、IL - 6、白血病抑制因子（LIF）、干细胞因子（SCF）或其合剂;

使活性成分的局部浓度高，其中活性成分含有药物或细胞因子、GM－CSF、IL－6、IL－2、IL－4或其合剂，当与辐射后肿瘤细胞或疫苗抗原（类似于用相应细胞因子基因转染的辐射后肿瘤细胞）一起用药时刺激保护性免疫反应；

诱发强免疫反应，其中活性成分包括如GM－CSF与抗原联合用药，特别是与肿瘤抗原、病毒抗原或细菌抗原；

局部注射组合物使伤口愈合，如其中活性成分包括GM－CSF；

引发抗原特异免疫的耐受性，其中活性成分为如GM－CSF与辅助分子（助受体）的抑制剂合用，CD28－B7相互作用、CD40－CD40配体相互作用、粘联因子相互作用的特别抑制剂；

与抑制细胞生长治疗联合治疗、或作为疫苗的辅剂，其中互相成分是如细胞因子，特别是细胞因子（IL－3、IL－6）或细胞因子分泌诱导剂如类脂衍生物，例如描述于EP 0309411，特别是在实施例1中的化合物，也称为MRL 953；

特殊的免疫抑制，如其中互相成分为亲免疫结合（immunophilinbinding）免疫抑制剂如环孢菌素（如环孢菌素A）、子囊霉素（如FK506）、或雷怕霉素（如WO 94/09010描述的雷怕霉素或其衍生物如40－O－羟乙基－雷怕霉素）；

通过抗炎细胞因子的缓释治疗或预防自身免疫性疾病和炎性疾病，如IL－6、IL－10或TGF β，或干扰素如IFN－β 1或Betaseron，或可溶性细胞因子受体或细胞因子受体拮抗剂如细胞因子IL－I、TNF α或IL－4；

通过IgE的高亲和力受体（FcERI）的可溶性α链的缓释治疗或预防过敏性疾病；

癌症治疗，如用 otreotide 、细胞因子特别是白细胞介素;

选择靶向，如用于治疗利什曼病、真菌感染、酶沉积病（泰－萨二氏病、高歇氏病）;

AIDS 或 ARC 治疗;

接种如用破伤风类毒素疫苗;

造血，例如其中的活性成分是红细胞生成素;

对炎症关节进行关节间注射，其中活性成分为抗炎药，优选口服不能生物利用或有非常短的半衰期如 IL － 1 β转化成酶抑制剂、含金属蛋白酶抑制剂。

一种提高哺乳动物对疫苗的免疫反应的方法，包括给需要接种的哺乳动物使用有效剂量的 GM － CSF 和疫苗，这描述于国际 PCT 申请 WO 94/01133 。但是，不能按照本发明的方式较好地阻止 GM － CSF 释放，在较长的时期内活性化合物的释放接近常数，通过这种方法重复使用 GM － CSF 的次数可以降低。

本发明特别提供了一种存在于聚合物中的药物活性化合物的药物组合物，它表现为非水解表面溶蚀，用于白细胞介素或 CSF 的非肠道给药，特别是存在于本文定义的聚合物中。

本发明也提供可一种给患者使用此类组合物的方法，包括为需要此治疗的患者非肠道给药。

本发明的贮库制剂可用于引人的特殊药物化合物，治疗其已知症状。

所用药物化合物及贮库制剂的准确量依赖于若干因素如所治疗的疾病、所需的治疗时间、药物化合物的释放速率及聚（碳酸亚乙酯）的降解性。

所需制剂可用已知方式制备。所需药物活性制剂的量及其释放速率可根据已知的技术在体外或体内测定，如特别活性制剂的血浆浓度保持在可接受水平的时间。基质的降解性也可通过体外或优选体内的技术测定，例如在特定的时间后测定皮下组织中基质物质的量。

本发明的贮库制剂可用如微粒的形式通过口、鼻或肺给药，优选皮下、肌注或静脉给药，特别是在适当液体载体中以混悬液或植入片的形式（如皮下）。

如果聚合物基质在 1 、 2 或 3 星期或 1 个月后充分降解，则本发明贮库制剂的重复给药会起作用。

本发明的聚（碳酸亚乙酯）基质的优点是在药物化合物释放期间聚合物链降解为小分子部分，由体液将它从用药位置转运走。

含有优选的化合物 octreotide 药物的实例为治疗肢端肥大症，在含有微粒的非肠道液体贮库制剂中肽占（共）聚合物基质重量的至少 0.1（优选 0.5 ）至 20 %，优选 2.0 至 10，特别优选 3 至 6 %。治疗 1 个月所需 octreotide 的总量在肢端肥大症中为 20 至 30mg 、在乳腺癌中达到 100 至 200mg 。

肽从微粒中释放的时间可以是 5 天至约 2 个星期或更长。

一般缓释制剂包含（共）聚合物载体中的 octreotide，当给兔或鼠使用剂量为 2mg/kg 动物体重的 octreotide，在较长的时间内 octreotide 的血浆浓度至少为 0.3ng/ml 并优选小于 20ng/ml 。

本发明药物组合物可含有其它添加剂，它也优选置于（共）聚合物中如自由基清除剂，特别是过氧自由基阴离子 O_2^- 的清除剂。这种清除剂（甲萘醌或维生素 C ）的存在降低了聚（碳酸亚乙酯）的降解速率（图 7 ）。

添加剂的另一种类型是羟基自由基的清除剂，羟基自由基可能在过

氧自由基阴离子 O_2^- 的影响下产生，例如多元醇，特别是糖醇，尤其是甘露醇。此添加剂对试验动物的体重也有有利的影响，此动物所用的药是微胶囊 IL - 3 。如果没有这种添加剂，会延缓体重的增加。当组合物是微粒形式时同一添加剂或其它添加剂可加入到微粒的外部，因为它对微粒悬液的稳定性有有利的影响，可防止絮状物和沉淀物的产生。

如果存在添加剂，其含量优选占制剂总重量的 1 至 90 % 。

过氧自由基阴离子 O_2^- 的影响有利于体外和体内的物质降解，如图 8 所示。残余物质的降解曲线接近直线并有不同的斜率，这是因为体内及体外的降解条件不同。每单位时间降解物质的量几乎是常数。

药物活性物质（如人 IL - 3 ）在过氧自由基阴离子 O_2^- 的影响下，它在体内释放的曲线与降解曲线一样接近直线（图 10 ），这意味着单位时间内释放的药物化合物的量几乎是常数。

图 11 记录了体内人 IL - 3 的释放和体内物质降解，表明体内物质降解和药物释放的相互关系为 1： 1 。

实施例 1 - 5 ：使用由二乙基锌和水制备的催化剂合成聚（碳酸亚乙酯）的一般方法

特定试验中反应物、溶剂、催化剂等的量见表 1 。

将 200ml 干燥二□恶烷及 19.5g （ 158mmol ）二乙基锌置于氮气氛下的 750ml 烧瓶中。烧瓶装有机械搅拌器、滴液漏斗、温度计和一个氮气入口。滴液漏斗上装有二氯化钙管。在冰浴中将溶液冷却至 10 ℃并慢慢加入存在于二□恶烷中的水 2.7ml （见表 1 ），保持温度在 10 - 15 ℃。在室温将反应混合物再搅拌 45 分钟，直到无色溶液变为浅黄色。将催化剂溶液转移至高压釜中，用 40g 二氧化碳处理并在 125 ℃加热表 1 中指明的时间。然后将混合物冷却至室温并加入 560g(12.7mol) 二氧化碳，

随后在 1 小时内慢慢加入 132g(3mol)环氧乙烷。反应进行的时间见表 1 。此后，在几小时内慢慢降低压力。用二 恶烷稀释粘浆状产品，将二α恶烷溶液到进 0.25M 的氯化氢水溶液中形成沉淀。将沉淀溶于适量的二氯甲烷（ 2 - 4 升）中，用 0.5M HCl 水溶液（ 2x ）和水（ 1x ）洗涤。用无水硫酸钠干燥溶液并根据溶液的粘度将溶液蒸发至 0.5 至 1.5 升。将二氯甲烷溶液到入 4 倍体积的甲醇中沉淀产品。过滤白色产品并在 0.5 毫巴/50 ℃干燥过夜。用丙酮再沉淀粗产品使其进一步纯化。由于碳酸亚乙酯的含量不同，故除了在 3.65 、 3.73 、 4.29 及 4.37ppm 的信号相对强度外所有的产品有相同的 1H-NMR 谱。

表 1： 制备聚（碳酸亚乙酯）类试验

实施例	环氧乙烷		CO_2	$Zn(C_2H_5)_2$二α恶烷	温度	时间
	[mol]	[mol]	[mmol]	[ml]	[℃]	[h]
1	3	13.6	158	300	50	64
2	3	9.1	158	500	20	64
3	3	13.6	158	300	20	240
4	3	13.6	158	300	20	40
5	3	13.6	158	300	20	22
6	3	13.6	238	300	50	64

所有试验在 1.0 升高压釜 NB2 中进行。所有试验中水：二乙基锌摩尔比为 0.95 。催化剂用 40g 二氧化碳在 125 ℃预处理 1 小时，实施例 1 除外（ 10 小时）。

表 2： 合成聚（碳酸亚乙酯）类的部分物理性质

实施例	Mw [kDa]	Mn [kDa]	Mw/Mn	Tg [°C]	η_{inh} [dl/g] 在 $CHCl_3$ 中 a)	碳酸亚乙酯含量(%)
1	141.9	32.2	4.40	19.3	0.6087	
2	627.3	133.5	4.70	23.5	1.4691	
3	477.0	83.6	5.71	18.7	1.2791	
4	758.0	97.5	7.77	20.6	1.7590	
5	721.6	80.7	8.95	22.9	2.44 b)	90
6	310.9	103.1	3.02	20.1	88	

a) 如果不特别指明，则在 20 ℃浓度为 10mg/ml

b) 浓度为 1mg/ml

实施例 7 - 11：使用由二乙基锌和二醇制备的催化剂合成聚（碳酸亚乙酯）类的一般方法

1. 催化剂的制备

将 200ml 干燥二 α恶烷置于氮气氛下 750ml 干燥的四颈烧瓶中。通过玻璃注射管加入 19.50g （ 158mmol ）二乙基锌。烧瓶装有机械搅拌器、滴液漏斗、温度计及氩气入口。滴液漏斗中装有 100ml 干燥二 α恶烷并装有氯化钙管。然后将仪器置于氩气气流中。在氩气流中， 9.00(145 mmol, 0.92 克分子当量) 的新蒸馏的、干燥的甘醇加入（在分子筛上）到滴液漏斗中的二 α恶烷中。机械搅拌烧瓶，在氩气氛下用冰浴将温度降低至 10 ℃。在 30 分钟内将甘醇的二 α恶烷溶液滴加至搅拌的二乙基锌的二

烷溶液中，这期间保持温度在 10 - 14 ℃。加入 1， 2 －亚乙基二醇溶液的同时观察到乙烷气体的放出和沉淀的产生。加毕移去冰浴并将混合物再搅拌 60 分钟，同时使其升至室温。然后在氩气氛将多相混合物转移至高压釜（ 1 升高压釜 NB2 ）中。在搅拌的同时，高压釜充入约 40g （ 0.9 mol ）二氧化碳并在 125 ℃加热 1 小时，用二氧化碳预处理催化剂。

2. 聚合反应

将装有预处理的催化剂的高压釜冷却至室温并再充入 560g （ 12.7 mol ）二氧化碳。然后在 1 小时内通过慢慢注射将 132g （ 3 mol ）环氧乙烷（ 99.8 % ）加入到高压釜中的搅拌的混合物中。加毕，将高压釜加热至表 3 中指明的温度并在此温度在指定的时间内搅拌混合物。

3. 后处理

将高压釜冷却至室温并慢慢将压力降至常压。用 7 升二氯甲烷吸收白色粘浆状产品，加入 1035ml 0.4M HCl 溶液，在室温将混合物搅拌 3 小时。将两相分离并将有机层用 3 升 0.5M HCl 洗涤两次，用 4.5 升水洗涤两次。再用 120 g 硫酸钠干燥二氯甲烷溶液并浓缩至最后体积为约 2 升。将此溶液慢慢加入 6 升甲醇中沉淀产品。在 40 ℃真空干燥沉淀 16 小时得到粗品聚合物，在按以下方法进一步纯化:

将粗品溶于二氯甲烷中，在 15 分钟内将溶液到入 5 倍体积的丙酮中沉淀产品。在 40 ℃真空干燥沉淀 16 小时得到相应的聚（碳酸亚乙酯）。产品的物理性质列于表 4 。所有产品在 1750 和 1225cm-1 有强 IR 吸收。碳酸亚乙酯单元的 1H - NMR 信号在 4.37 ppm 。

表 3： 使用由二乙基锌和二醇合成聚（碳酸亚乙酯）类

实施例	环氧乙烷 Oxide [mol]	CO_2[mol]	溶剂 [a] [ml]	$(C_2H_5)_2Zn$ [mmol]	二醇 [b] [mmol]	反应温度 [°C]	反应时间 [hrs]
7	3,0	13,6	300	158	145	20	96
8	3,0	13,6	300	158	145	50	96
9	3,0	13,6	300	158	145	60	96
10	3,0	13,6	300[c]	158	145	50	144
11	3,0	13,6	300	158	145[d]	50	96

a) 二口恶烷（如不特别指明）

b) 甘醇（如不特别指明）

c) 用四氢呋喃代替二口恶烷作为溶剂

d) 用 1， 4 －丁二醇代替甘醇

表 4： 用由二乙基锌和二醇制备的催化剂合成的聚（碳酸亚乙酯）类的部分物理性质

实施例	Mw [kDa]	Mn [kDa]	Mw/Mn	Tg [°C]	η_{inh}[dl/g] 在 $CHCl_3$ 中 [a]	碳酸亚乙酯含量(%)
7	-	-	-	16.7	2.88 [b]	98
8	328.0	149.0	2.20	16.4	0.97	95
9	207.0	103.0	2.00	21.2	0.65	92
10	231.0	83.8	2.76	32.6	0.72	96
11	110.0	53.4	2.06	31.1	0.49	90

a) 如不特别指明，则在 20 ℃且浓度为 10mg/ml

b) 浓度为 1 mg/ml

实施例 12 ： 使用由二乙基锌和间苯三酚制备的催化剂合成聚（碳酸亚乙酯）的试验方法

1. 催化剂的制备

将 200ml 干燥二 α恶烷置于氮气氛下 750ml 干燥的四颈烧瓶中。通过玻璃注射管加入 19.60g （ 158.7mmol ）二乙基锌。烧瓶装有机械搅拌器、滴液漏斗、温度计及氩气入口。滴液漏斗中装有 100ml 干燥二 α恶烷并装有氯化钙管。然后将仪器置于氩气气流中。在氩气流中， 13.34(105.8 mmol, 0.92 克分子当量) 干燥的间苯三酚加入（在分子筛上）到滴液漏斗中的二 α恶烷中。机械搅拌烧瓶，在氩气氛下用冰浴将温度降低至 10 ℃。在 30 分钟内将间苯三酚的二 α恶烷溶液滴加至搅拌的二乙基锌的二α恶烷溶液中，这期间保持温度在 10 - 14 ℃。加入间苯三酚溶液的同时观察到乙烷气体的放出和沉淀的产生。加毕移去冰浴并将混合物再搅拌 30 分钟，同时反应升至室温。然后在氩气氛将多相混合物转移至高压釜（ 1 升高压釜 NB2 ）中。在搅拌的同时，高压釜充入约 40g （ 0.9 mol ）二氧化碳并在 125 ℃加热 1 小时，用二氧化碳预处理催化剂。

2. 聚合反应

将装有预处理的催化剂的高压釜冷却至室温并再充入 560g （ 12.7 mol ）二氧化碳。然后在 1 小时内通过慢慢注射将 132g （ 3 mol ）环氧乙烷（ 99.8 % ）加入到高压釜中的搅拌的混合物。加毕，高压釜在 21 ℃搅拌 260 小时。

3. 后处理

将高压釜冷却至室温并慢慢将压力降至常压。用共 4 升二氯甲烷吸收白色粘浆状产品，加入 1035ml 0.4M HCl 溶液，在室温将混合物搅拌 3 小时。将两相分离并将有机层用 1.5 升 0.5M HCl 洗涤两次，用 2 升水洗涤两次。再用 120 g 硫酸钠干燥二氯甲烷溶液并浓缩至最后体积为约 1 升。将此溶液慢慢加入 3 升甲醇中沉淀产品。在 40 ℃真空干燥沉淀 16 小时得到粗品聚合物，在按以下方法进一步纯化:

将粗品溶于二氯甲烷中，在 15 分钟内将溶液到入 5 倍体积的丙酮中沉淀产品。再将沉淀溶于二氯甲烷中，从甲醇中再沉淀并在 40 ℃真空干燥沉淀 16 小时得到相应的聚（碳酸亚乙酯）。

产品的物理性质:

Mw=258000 Da, Mn=35600 Da, Tg=15.4 ℃。

IR：在 1751 和 1225cm-1 强吸收。

根据 1H - NMR，产品的碳酸亚乙酯含量为 96 %。

实施例 13：使用由二乙基锌和丙酮制备的催化剂合成聚（碳酸亚乙酯）的试验方法

132g(3 mol)环氧乙烷与 600g （ 13.6mol ）二氧化碳在 50 ℃使用由 8.43g （ 145.16 mmol ）丙酮和 19.62g （ 159mmol ）二乙基锌制备的催化剂共聚反应 96 小时。

除了用丙酮代替二醇制备催化剂外，催化剂的制备和聚合反应按照与实施例 7 - 11 相似的方法进行。

这样制得的聚（碳酸亚乙酯）的碳酸亚乙酯含量为 93 % 并具有如下性质:

Mw=233kDa, Mn=109kDa, Mw/Mn=2.14, Tg=22.4 ℃。

实施例 14： 合成末端基团硬脂酰化的聚（碳酸亚乙酯）

将 1 g 聚（碳酸亚乙酯）（ Mw=153000 Da, Mn=68900 Da, Tg=29.1 ℃。）溶于 30 ml 干燥的二氯甲烷中。随后用 0.98g （ 12.38 mmol ）吡啶和 10 g （ 33.0 mmol ）硬脂酰氯处理溶液。在室温将反应混合物搅拌 48 小时，然后用 50 ml 二氯甲烷稀释并用 2x150ml 饱和碳酸氢钠和水连续洗涤。用无水硫酸钠干燥有机相，通过将二氯甲烷溶液滴加进 300 ml 正己烷中沉淀产品。这样得到的粗品通过溶于二氯甲烷并从 3 倍体积的乙醚中沉淀进一步纯化。最后，在 40 ℃真空干燥产品 16 小时得到末端基团硬脂酰化的聚（碳酸亚乙酯）。

Mw=144000 Da, Mn=71000 Da, Tg=25.6 ℃。

实施例 15： 末端基团乙酰化的聚（碳酸亚乙酯）的合成

将 1 g 聚（碳酸亚乙酯）（ Mw=153000 Da, Mn=68900 Da, Tg=29.1 ℃。）溶于 10 ml 干燥的二氯甲烷中。加人 0.98g （ 12.38 mmol ）吡啶，随后加人 10.08 g （ 98.7 mmol ）乙酸酐。在室温将反应混合物搅拌 120 小时，然后用 50 ml 二氯甲烷稀释并缓慢加人到 200 ml 饱和碳酸氢钠中。混合物搅拌 30 分钟然后分离两相溶液。再用 150ml 饱和碳酸氢钠和最后用水洗涤有机相。用无水硫酸钠干燥二氯甲烷溶液，通过将此溶液滴加进 300 ml 乙醚中沉淀产品。沉淀再溶于二氯甲烷并从乙醚中再沉淀。在 40 ℃真空干燥产品 16 小时得到具有末端乙酸酯基团的聚（碳酸亚乙酯）。

Mw=150000 Da, Mn=69100 Da, Tg=26.8 ℃。

实施例 16： 通过用沸水处理纯化聚（碳酸亚乙酯）

将 1 g 聚（碳酸亚乙酯）（实施例 8， Mw=328000 Da, Mn=149000 Da, Tg=16.4 ℃。）切割成小片并在沸水中搅拌 2 小时. 除去水并换成新鲜

水，再将其加热至沸点。 3 小时后，分离聚合物片并在 40 ℃真空干燥 16 小时。得到的产品具有以下物理性质： Mw=340000 Da, Mn=148000 Da, Tg=28.3 ℃。这样，在不改变聚合物分子量的前体下，观察到玻璃化温度急剧增加。

实施例 17： 含 1 % hIL-3 药物填料的组合物（微粒）

1. 含微粒药物的制备

将 1g 聚（碳酸亚乙酯）（实施例 8 （ PEC ） Mw=328000 ）边搅拌边溶于 10ml 二氯甲烷，随后加入溶于 0.6ml 水中的 12.1mg 人白细胞介素 3 （ hIL-3 ）。用 Ultra-Tarrax 在 20000 rpm 彻底搅拌混合物 1 分钟（ = 内 W/O - 相）。在 50 ℃将 1 g 明胶 A 溶于 2000ml 去离子水中并将溶液冷却至 20 ℃（外 W 相）。彻底搅拌 W/O - 相及 W - 相。这样内 W/O - 相均匀分散在外 - W - 相中形成细的小滴。所得三相乳液缓慢搅拌 1 小时。以此将二氯甲烷蒸发，由内相的小滴形成微粒并硬化。

微粒沉积后，吸除上清液并通过真空过滤或离心回收微粒，用水清洗除去明胶。最后，微粒通过以甘露醇作填充剂冷冻干燥或在真空烘箱中干燥 72 小时（无甘露醇的制剂），过筛（筛目大小为 0.125mm ）得到最佳产品。

2. 空白对照剂

将 1g 实施例 8 的 PEC， Mw = 328000 边搅拌边溶于 10ml 二氯甲烷（内 0 - 相）。在 50 ℃将 1g 明胶 A 溶于 2000ml 去离子水中并将溶液冷却至 20 ℃（ = 外 W 相）。彻底搅拌 0 - 相及 W - 相。这样 0 - 相均匀分散在外 W - 相中形成细的小滴。所得乳液缓慢搅拌 1 小时并按上述方法处理。

实施例 18 - 26：

此后描述的所有明胶制剂是使用按照实施例 8 表 3 合成的 PEC 类制备并以类似于实施例 16 的方法纯化。它们的 Mw 为 300000 至 450000，碳酸亚乙酯的含量大于 94 %， Tg 为 18 至 50 ℃。

实施例 18： 含 0.2 % hIL-2 填料的组合物（微粒）

将 2.9mg 人白细胞介素 2 （ hIL-2 ）溶于 1.5ml 水中，并按照实施例 17 制备含 IL － 2 的微粒。微粒通过以甘露醇作填充剂冷冻干燥并过筛（筛目大小为 0.125mm ）得到最终产品。

实施例 19： 含 0.2% hIL-2 填料（无水）的组合物（微粒）

按照实施例 18 制备制剂，但不同的是将 2.9mg 人白细胞介素 2 直接分散在有机相中（ PEC 溶解于二氯甲烷）。

实施例 20： 含 0.8% hIL-3 填料的组合物（植入剂）

1. 压模

将含有 100 %（ w/w)聚(碳酸亚乙酯)(空白对照剂)、 99 %（ w/w ）聚（碳酸亚乙酯）和 1 %（ w/w ）人白细胞介素 3 或 79.2%（ w/w ）聚（碳酸亚乙酯）、 20 %（ w/w ）甘露醇及 0.8%（ w/w ）人白细胞介素 3 的微粒 25mg 在 60 － 70 ℃、 160 巴压模 3 分钟，制成直径 5 mm 的植入剂（片剂）。在用于体外或体内药物释放试验前，此片剂在 4 ℃装于密闭的玻璃瓶中。

2. 体外药物释放试验

无甘露醇白细胞介素 3 、含甘露醇白细胞介素 3 及空白对照剂的三种片剂 37 ℃在合成培养基中摇动。该培养基含有 2.5%（ v/v ） N －[2 －羟乙基] － 哌嗪 － N′ －[2 － 乙基磺酸]（ 1m ）、 10 %（ v/v ）小牛血清和 2 %（ v/v)青霉素/链霉素溶液。在第 0.5 、 1 、 2 、 5 小时及 1 、 2 、 3 、 7 、 14 、 20 小时从培养基中取样，随后更新培养基。样品中

人白细胞介素 3 的含量通过 ELISA 测定。

3. 体内药物释放试验

将最佳状态的雄性鼠通过吸入麻醉剂麻醉，将人白细胞介素 3 制剂和空白对照剂的片剂植入每个鼠的皮下囊中。在第 1 、 4 、 7 、 14 、 21 天后，通过吸入过量的麻醉剂将鼠处死。取出残留的片剂，除去附着的组织并干燥。通过差重法测定片剂的物质损失。随后，通过 HPLC 和 ELISA 测定残留片中人白细胞介素 3 的含量。

实施例 21： 含有 0.0002%-2% hIL-2 填料的组合物（ w/o/w 微粒）

将 4 gPEC 边电磁搅拌边溶于 80 ml 二氯甲烷中。向此溶液中加入溶于 6 ml 蒸馏水或含几滴乙醇的水中的适量的 IL - 2 （ 113.2 mg 对应 2 %， 11.32 mg 对应 0.2 %等）中。用 Ultra-Turax 彻底混合混合物，将 IL - 2 溶液分散于聚合物相（ = 内 W/O 相）中。在 50 ℃将 1 g 明胶 A 溶于 200ml 1/15M 磷酸盐缓冲液（ pH=7.4 ）中并将溶液冷却至 20 ℃（ = 外 W 相）。彻底混合 W/O - 相及 W - 相。这样内 W/O - 相均匀分散在外 - W - 相中形成细的小滴。所得三相乳液缓慢搅拌 1 小时。以此将二氯甲烷蒸发，由内相的小滴形成微粒并硬化。

微粒沉积（或离心）后，吸除上清液并通过真空过滤回收微粒，用水清洗除去明胶。最后，微粒在真空烘箱中干燥 24 小时，过筛得到最终产品。

用 HPLC 和生物试验测定的包囊效率为 10 至 100 %。

实施例 22： 含 0.0002%-2% IL-2 填料的组合物（ s/o/w 微粒）

按照实施例 21 制备制剂，不同的是 IL - 2 不是溶于水中。 IL - 2 不用溶解，药物直接分散到聚合物相（ = O - 相）中。用 HPLC 和生物试验测定的包囊效率为 10 至 100 %。

注意：聚合物、二氯甲烷、水及药物的量可在大范围内变化而不改变产品的性质。可制得较高的载药量为 20 %。在外相中，用聚乙烯醇等其它乳化剂代替明胶，乳化剂/缓冲剂的浓度也可变化。分离和干燥方法可用其它熟知的制药技术代替，如过滤、冻干或喷雾干燥。

实施例 23：含 1 % hGM-CSF 填料的组合物（ w/o/w 和 s/o/w 微粒）

按照实施例 21 和 22 的方法制备 s/o/w 和 w/o/w 制剂。但 w/o/w 制剂的包囊效率为 60 %，而 S/O/W 制剂具较低的包囊效率。

实施例 24：含 1 至 10 % Octreotide-双羟萘酸盐（ SMS - PA ）填料的组合物（ w/o/w 和 s/o/w 微粒）

按照实施例 19 和 20 的方法制备。但 SMS - PA 不溶于水。因此，对于 W/O/W 制剂药物是分散在而不是溶解在水中。通过 HPLC 测定的包囊效率为 20 至 100 %。

实施例 25：含 1 至 10 % Octreotide-乙酸盐填料的组合物（ w/o/w 和 s/o/w 微粒）

按照实施例 21 和 22 的方法制备。通过 HPLC 测定的包囊效率为 2 至 40 %，这明显比亲脂性的 SMS - PA 低。在使用冻干的活性化合物物质后，在 S/O/W 制剂中得到较高值（较小的药物颗粒）。

实施例 26：从兔体内微粒及兔和鼠体内植入剂中释放 Octreotide 双羟萘酸盐（ SMS - PA ）

给雄性兔（灰鼠杂种，体重约 3 kg ）皮下植入聚（碳酸亚乙酯）片或注射聚（碳酸亚乙酯）微粒（含药量 1.95%），用量约为 2 mg 药物每公斤体重。给雄性鼠（ Wistar，体重约 375 g ）皮下植入片。每个鼠和兔的量分别是 40 和 300 mg 含药的聚合物，以微粒的形式，将其分别压成植入剂或作为混悬剂使用。

鼠和兔的植入片的直径分别为 0.5 和 1 cm，并按照实施例 20 制备。

为测定药物释放，在第 14 和 21 天分别收集鼠和兔的血样，并通过放射免疫测定法及 HPLC 测定植入剂中的药物残余物。

可以发现正如高分子量物质 hIL - 3（图 11），聚（碳酸亚乙酯）的物质损失和 SMS - PA 的释放为直线关系（图 13）。给兔用药后 3 星期植入物质有最大的降解值 75 %，给鼠用药后 2 星期植入物质有最大的降解值 95 %。炎性反应（包括多形核白细胞及其它细胞的侵入）是聚（碳酸亚乙酯）发生生物降解的前提。可以预计炎性反应的过程是种特异的，引起药物血浆水平的种特异性现象。此发现针对 SMS - PA（图 12）。聚（碳酸亚乙酯）在鼠体内比在兔体内生物降解更快。兔的 SMS - PA 的血浆水平缓慢地增加，在约地 9 天达到恒定的释放状态，并持续 21 天。

实施例 27：含 0.0002%-2% rhIL - 6 填料的组合物（w/o/w 微粒）

将 4 gPEC 边电磁搅拌边溶于 80 ml 二氯甲烷中。向此溶液中加入溶于 6 ml 蒸馏水或含几滴乙醇的水中的适量的 rhIL - 6（113.2 mg 对应 2 %，11.32 mg 对应 0.2 % 等）。用 Ultra-Turax 彻底混合混合物，将 IL - 6 溶液分散于聚合物相（=内 W/O 相）中。在 50 ℃将 1 g 明胶 A 溶于 200ml 1/15M 磷酸盐缓冲液（ph=7.4）中并将溶液冷却至 20 ℃（=外 W 相）。彻底混合 W/O - 相及 W - 相。这样内 W/O - 相均匀分散在外 - W - 相中形成细的小滴。所得三相乳液缓慢搅拌 1 小时。以此将二氯甲烷蒸发，由内相的小滴形成微粒并硬化。

微粒沉积（或离心）后，吸除上清液并通过真空过滤回收微粒，用水清洗除去明胶。最后，微粒在真空烘箱中干燥 24 小时，过筛得到最终产品。

用 HPLC 和生物试验测定的包囊效率为 10 至 100 %。

实施例 28： 含 0.0002%-2% rhIL - 6 填料的组合物（ s/o/w 微粒）

按照实施例 27 制备制剂，不同的是 IL - 6 不是溶于水中。 IL - 6 不用溶解，药物直接分散到聚合物相（ = O - 相）中。用 HPLC 和生物试验测定的包囊效率为 10 至 100 %。

注意：聚合物、二氯甲烷、水及药物的量可在大范围内变化而不改变产品的性质。可制得较高的载药量为 20 %。在外相中，用聚乙烯醇等其它乳化剂代替明胶，乳化剂/缓冲剂的浓度也可变化。分离和干燥方法可用其它熟知的制药技术代替，如过滤、冻干或喷雾干燥。

实施例 29 - 31：使用 IL - 6 治疗 TNF α和/或 IL - 1 介导的疾病

实施例 29：多发性硬化的动物模型： Lewis 鼠试验诱发的过敏性脑脊髓炎模型的慢性复发（ CR - EAE ）

本领域试验诱发的过敏性脑脊髓炎（ EAE ）是人多发性硬化的很好的研究试验模型。[Paterson, ADV. IMMONOL. 5(1966) 131-208; Levine et al., AM. J. PATH. 47(1965) 61; Mcfarlin et. al., J. IMMUNOL. 113(1974) 712; Borel, TRANSPLANT & CLIN. IMMUNOL. 13(1981)3]。给鼠注射其它种的神经组织和佐剂，用所得过敏反应导致的鼠神经损伤模拟多发性硬化产生的自身免疫损伤。鼠变得部分或完全麻痹，测定用药或不用药时疾病的严重程度。一些药物如甾类和免疫抑制剂有减慢疾病发作的活性，但一旦染上疾病它们不能预防其复发。

因此，试验诱发的过敏性脑脊髓炎模型的慢性复发（ CR - EAE ）[Feurer, et. al., J. NEUROIMMUNOL: 10(1985)159-166]被看作特别符合需要的模型，它能非常接近地模拟在治疗多发性硬化患者中的实际困难。在此模型中，疾病是通过注射豚鼠脊髓和富含结核分支杆菌的福氏佐剂诱发的。一般 75 - 80 %的被致敏鼠感染 CR - EAE，在开始的 40

天里复发 2 - 3 次。 60 - 80 天后，约 50 %患 CR - EAE 鼠进一步复发，所有病例中完全恢复率只有 35 %。其余的 65 %疾病进一步发展。在从第一次疾病发作恢复后，第 16 天开始药物治疗。

重组人白细胞介素 6 （ rhIL-6, Sandoz ）溶于生理盐水中，在第 16 天开始用 10 微克 IL - 6 （约 50 μ g/kg ）隔天每只鼠腹膜内注射。对照组和 IL - 6 用药组在第 11 - 14 天有普遍的严重疾病发作（急性）。严重程度的评分为 0 = 无疾病至 4 = 动物完全麻痹，对照组平均为 3.0 而 IL - 6 组平均 3.2 。从第 16 天至第 30 天（共 7 次给药）隔天使用 IL - 6 使疾病几乎完全抑制。在第 16 天后， 5 只对照鼠全部第二次发作，其平均严重发病率为 1.8 ，在第 22 - 29 天第三次发作。在 IL - 6 用药组中未观察到其它发作。

实施例 30： 关节炎动物模型: 对患严重综合免疫缺乏（ SCID ）鼠由疏螺旋体属诱发的关节炎

莱姆关节炎（或莱姆病关节炎）代表了一类独特的慢性关节炎，因为诱发因素已知是必然的。此病的主要特征是由蜱生螺旋体疏螺旋体属 burgdorferi 感染诱发。莱姆病关节炎病人滑膜损伤特征与类风湿关节炎患者的滑膜非常接近。在两组病人中，观察到滑膜衬里细胞 hyperthrophy 、滑膜细胞增生、血管增殖及在滑膜衬里区域单核细胞侵润。发现很多浆细胞、高内皮小静脉、散布巨噬细胞及很少的树突细胞有强 MHC II 类抗原表达。此外，在多种关节炎疹患者的滑膜液中发现了 IL - 1 、 IL - 6 和 TNF α等细胞因子，这表明这些细胞因子可能与关节损伤的病因有关。最近，用缺乏功能性 T 和 B 细胞的 SCID 鼠建立了莱姆病关节炎的鼠模型（ M. M. Simon, et. al. (1990) Immunology Todday 12: 11 ）。用疏螺旋体属 burgdorferi 感染免疫缺乏的鼠导致明显的且

持续的低关节炎。疏螺旋体属诱发 SCID 鼠关节炎，对皮质甾类（ 30mg/kg 皮下使用强的松）有反应，而对剂量达 30mg/kg s.c. 如 SIM （环孢菌素 A ）等免疫抑制剂无反应。这是细胞因子造成的（也包括诱发因素已知确定的其它类型）关节炎的较好的模型。

通过尾底部注射给 6 星期大的 C.B - 17 SCID 鼠（ SCID 突变的纯合子，得自 Bomholtgard, Denmark, 5-6 只动物/组）接种 100 mio. 疏螺旋体属 burgdorferi 有机体。有免疫能力的 C.B - 17 鼠（相同来源）作为对照动物。注射疏螺旋体属 burgdorferi 后它们不得任何疾病。用生理盐水稀释重组人 IL - 6 （ rhIL-6, Sandoz, 保存液浓度为 5mg/ml ），并每星期 5 次给药，共腹膜内注射 17 次，剂量为 10 微克每只鼠。以双盲形式每天观察小鼠胫跗及尺腕关节的关节炎临床病症。按照下列参数临床评分:

- \- 无病症
- ? 有疑问
- (+) 关节变红
- \+ 轻度肿胀
- + + 中度肿胀
- + + + 胫跗和尺腕关节严重肿胀。

在临床关节炎的高峰值，杀死小鼠，用 Schaffer 氏溶液固定关节，置于 9100 塑料中并用苏木紫伊红染色。

组	第 n 天的临床病症（肿胀的关节数/关节总数）					
	13	14	15	16	17	20
对照	0/30	0/30	0/30	0/30	0/30	0/30

SCID、IL-6	6.5/36	12.5/36	15/36	21/36	30/36	35/36
% 患关节炎	18 %	35 %	42 %	58 %	83 %	97 %
SCID、IL-6 治疗	4/30	3.5/30	11/30	7.5/30	12/30	10.5/30
% 患关节炎	13 %	12 %	37 %	25 %	40 %	35 %

未用 IK - 6 治疗的 SCID 小鼠因疏螺旋体属 burdorferi 感染在抗原注射约第 13 天后产生严重的关节炎。在所有病鼠中，低剂量的 rhIL-6 以平均 60 - 75 %降低关节炎的严重程度。

实施例 31：败血症休克的鼠模型

因为其广泛地用作人败血症休克的模型，故决定在小鼠内毒素休克模型中使用 d - 半乳糖胺致敏小鼠研究 IL - 6 的效果。我们的方法和结果如下:

雌性 OF1 小鼠重 18 - 22g，腹膜内注射含 0.15 mg/kg 脂多糖内毒素（ LPS ）和 500 mg/kg d - 半乳糖胺的 PBS 溶液 0.2 ml 。将每 10 只小鼠分为一组，并作如下处理:

实验 1

时间	11:00	14:00	16:00
组 1	PBS	LPS + d-GAL	PBS
组 2	IL-6 (50 μg)	LPS + d-GAL	PBS
组 3	PBS	LPS + d-GAL + IL-6 (50 μg)	PBS
组 4	PBS	LPS + d-GAL	IL-5 (50 μg)

实验 2

时间	11:00	14:00	16:00
组 1	PBS	LPS + d-GAL	PBS
组 2	IL-6 (50 μg)	LPS + d-GAL	PBS
组 3	PBS	LPS + d-GAL + IL-6 (100 μg)	PBS

组 4	PBS	LPS + d-GAL + IL-6 (20 μg)	PBS
组 5	PBS	LPS + d-GAL + IL-6 (5 μg)	PBS
组 6	PBS	LPS + d-GAL + IL-6 (0.8 μg)	PBS
组 7	PBS	LPS + d-GAL + IL-2 (100 μg)	PBS
组 8	PBS	LPS + d-GAL + IL-4 (50 μg)	PBS
组 9	PBS	LPS + d-GAL	IL-6 (50 μg)

rhIL-6 （ ILS 969, Sandoz ）、 rhI1-2 （ Sandoz ）及 rhIL-4 （ Sandoz ）用 PBS 稀释。所有注射（体积 0.2 ml ）为腹膜内注射。在组 3 （试验 1 ）和组 3 至 8 （试验 2 ） IL - 6 和 IL - 2 稀释人 LPS/d-GAL 溶液中，以便小鼠得到单剂量 0.2 ml 注射。括号中的数字表示每只小鼠白细胞介素的用药量。需要多剂量 PBS 控制由于应激反应产生的组间变化性，此应激反应因在 LPS 使用前或后的不同时间的操作产生。

观察小鼠存活 48 小时。我们使用卡方检验进行统计计算。如图 1 所示，在使用 LPS 24 小时后 10 只对照小鼠中有 9 只死亡。在 LPS 注射前 3 小时或后 2 小时用 IL - 6 治疗分别将死亡率降低至 60 % （ p=0.12 ）和 70 % （ p=0.26 ）。另一方面，在使用 LPS 同时使用 IL - 6 将死亡率降低至 10 % （ p=0.01 ）。保护效果持续很长时间，因为 48 小时后组 3 中的死亡率只轻微增加（即增至 30 % ），与对照组相比仍有显著的保护（ p<0.01 ）。组 4 的死亡率在 70 % 至 80 %，而在组 1 和 2 中未观察到

变化.

基于这些结果，我们试验不同剂量 IL - 6 的效果。在注射 LPS 的同时使用 IL - 6 ，因为按照第一个试验这是最佳的时间。我们也在使用 LPS 时测定 IL - 3 和 IL - 4 的效果，以此排除由于在 LPS/d-GAL 制剂中使用重组蛋白质带来的可能的人为因素干扰。我们也试验了在注射 LPS 前或后使用剂量为 100 μ g/每只小鼠的 IL - 6 是否能防止内毒素死亡。

试验 2 的结果（图 2 ）与试验 1 的一致。在此试验中，也用 IL - 6 防止小鼠由于内毒素死亡。当 IL - 6 与 LPS 一起使用时，注射 LPS 后 24 小时的保护结果依赖于剂量：剂量为 20 、 4 、 0.8 μ g/每只小鼠时，死亡率分别为 30 % (p=0.03)、 50 % (p=0.16)、 70 % (p=0.61)，而剂量为 100 μ g/每只小鼠（死亡率 60 %， p=0.33 ）比剂量 20 μ g/每只小鼠的保护效果差。 LPS 注射前或后使用 100 μ g/小鼠与相同剂量和 LPS 一起使用所观察到的保护结果相当。在注射 LPS48 小时后的存活率与前面的相似。

在注射 LPS 时使用 IL - 4 对防止小鼠因内毒素死亡无效，而 IL - 2 会降低小鼠的存活率。

图1/13 链霉蛋白酶对聚（碳酸亚乙酯）体外物质降解的影响

（仪器 +/-扫描电子显微镜； n=3）

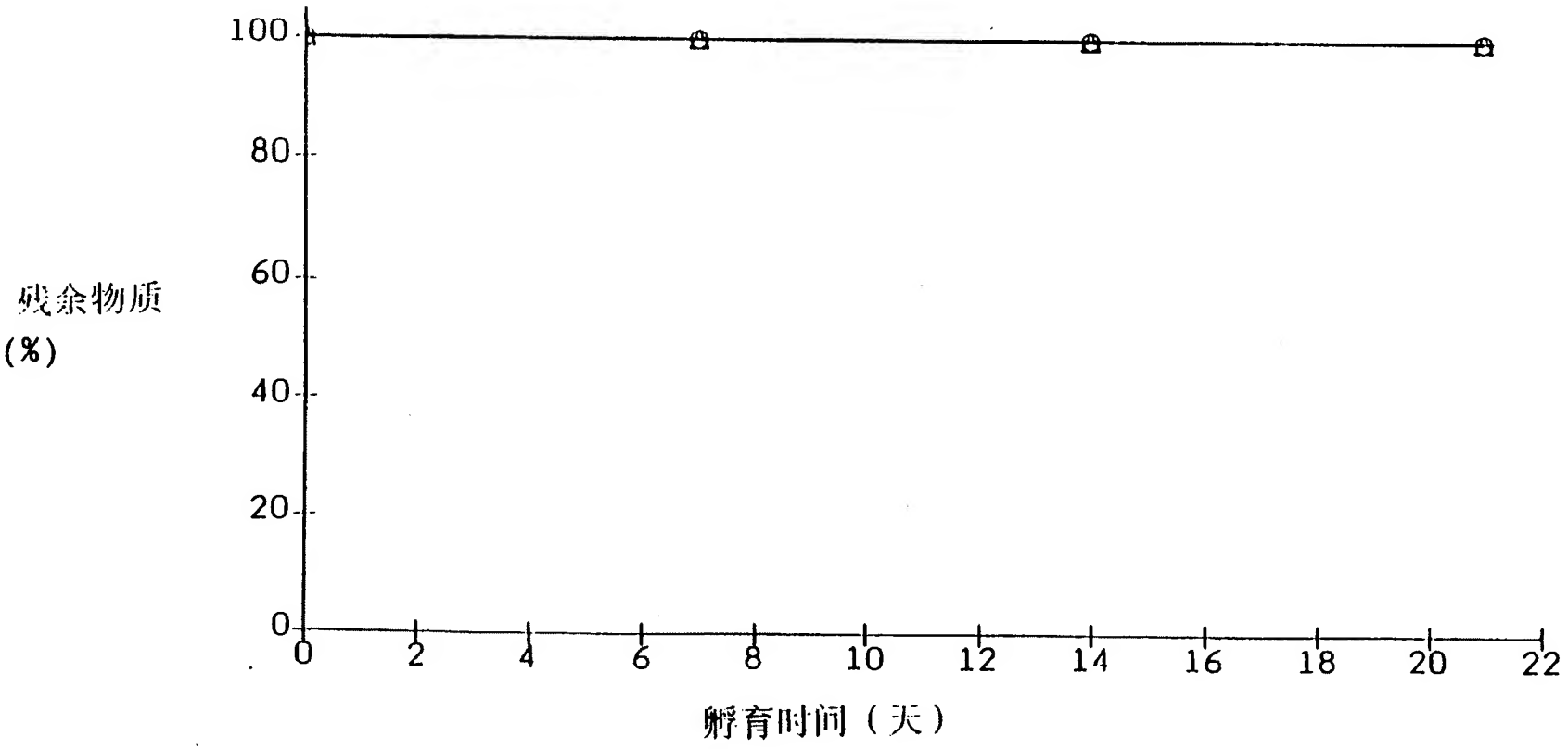


图2/13　pH 7.4磷酸生理盐水缓冲液（PBS）中聚（碳酸亚乙酯）植入剂的膨胀

（仪器 +/-扫描电子显微镜；n=3）

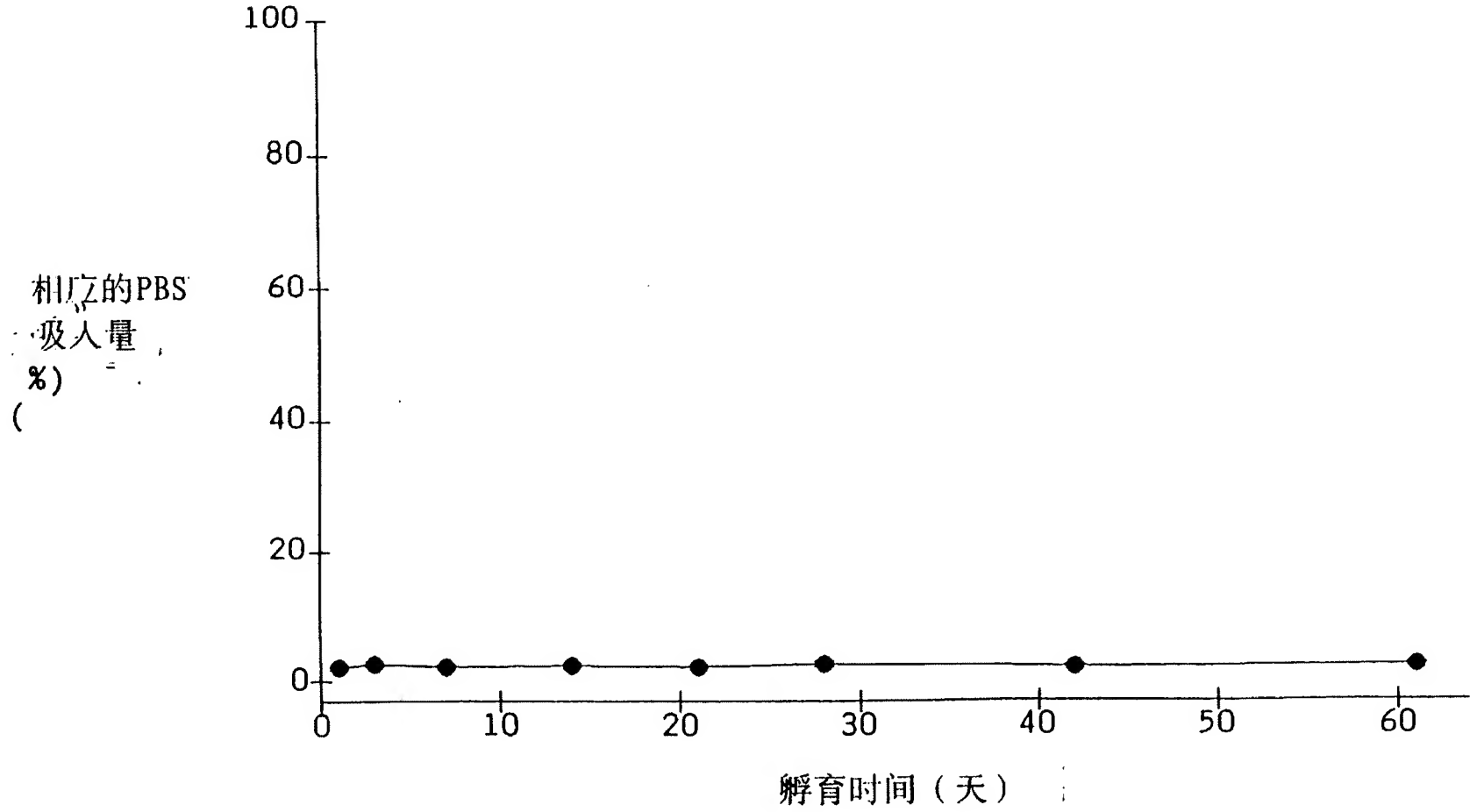
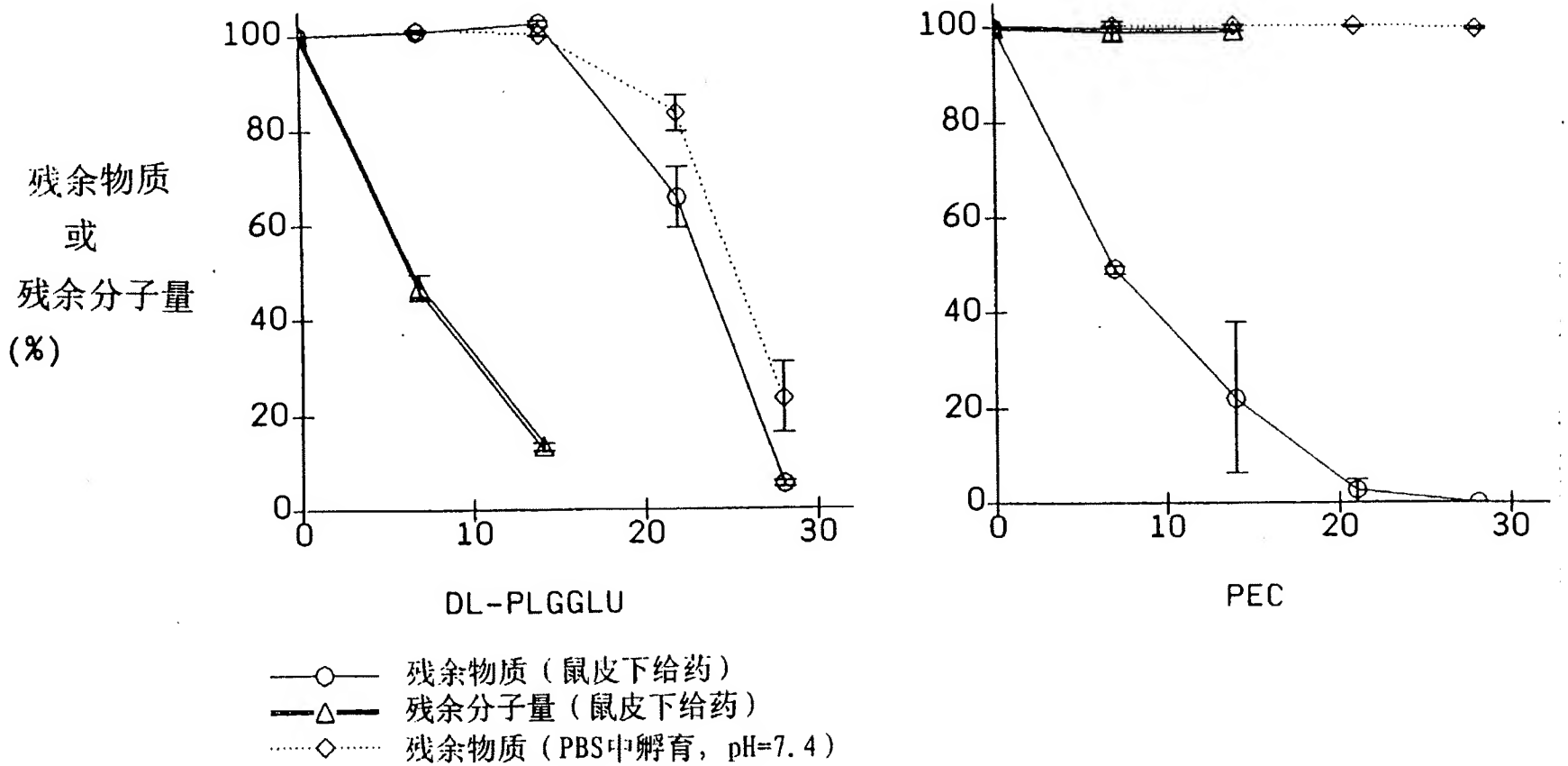


图3/13　D－葡萄糖引发的聚（DL－乙交酯－丙交酯）共聚物（DL－PLGGLU）（54.6：45.4）的水解整体溶解和聚（碳酸亚乙酯）（PEC）的非水解表面溶蚀（仪器 +/-扫描电子显微镜；n=3）



下[illegible]鼠体的聚(碳酸亚乙酯)[illegible]

[illegible]皮[illegible]于[illegible]

[illegible]质[illegible]扫描电子显微镜; n=3)

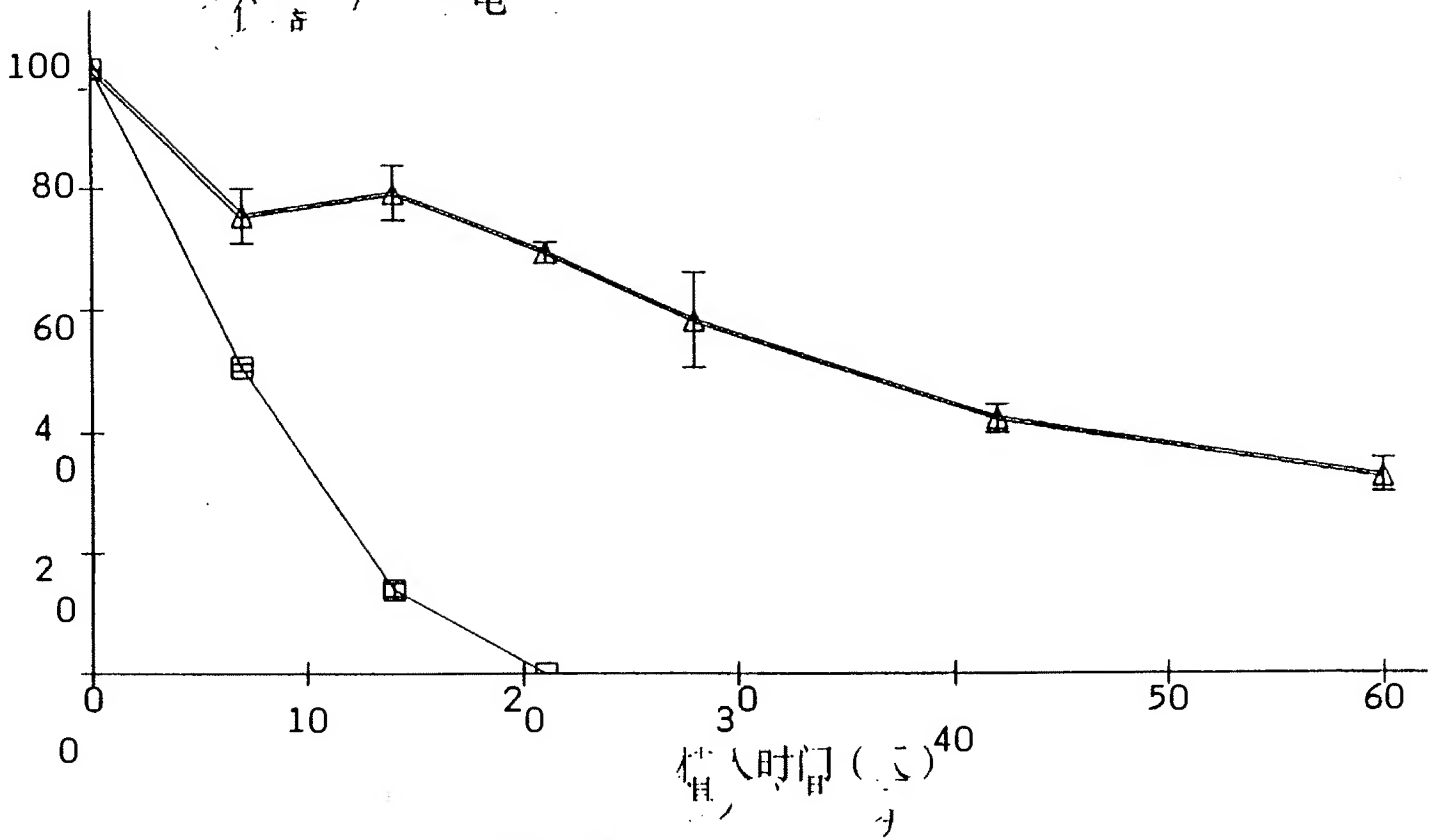


图5/13　多核细胞中聚（碳酸亚乙酯）诱发的过氧化物（细胞色素 C试验）

（仪器 +/-扫描电子显微镜；n=3）

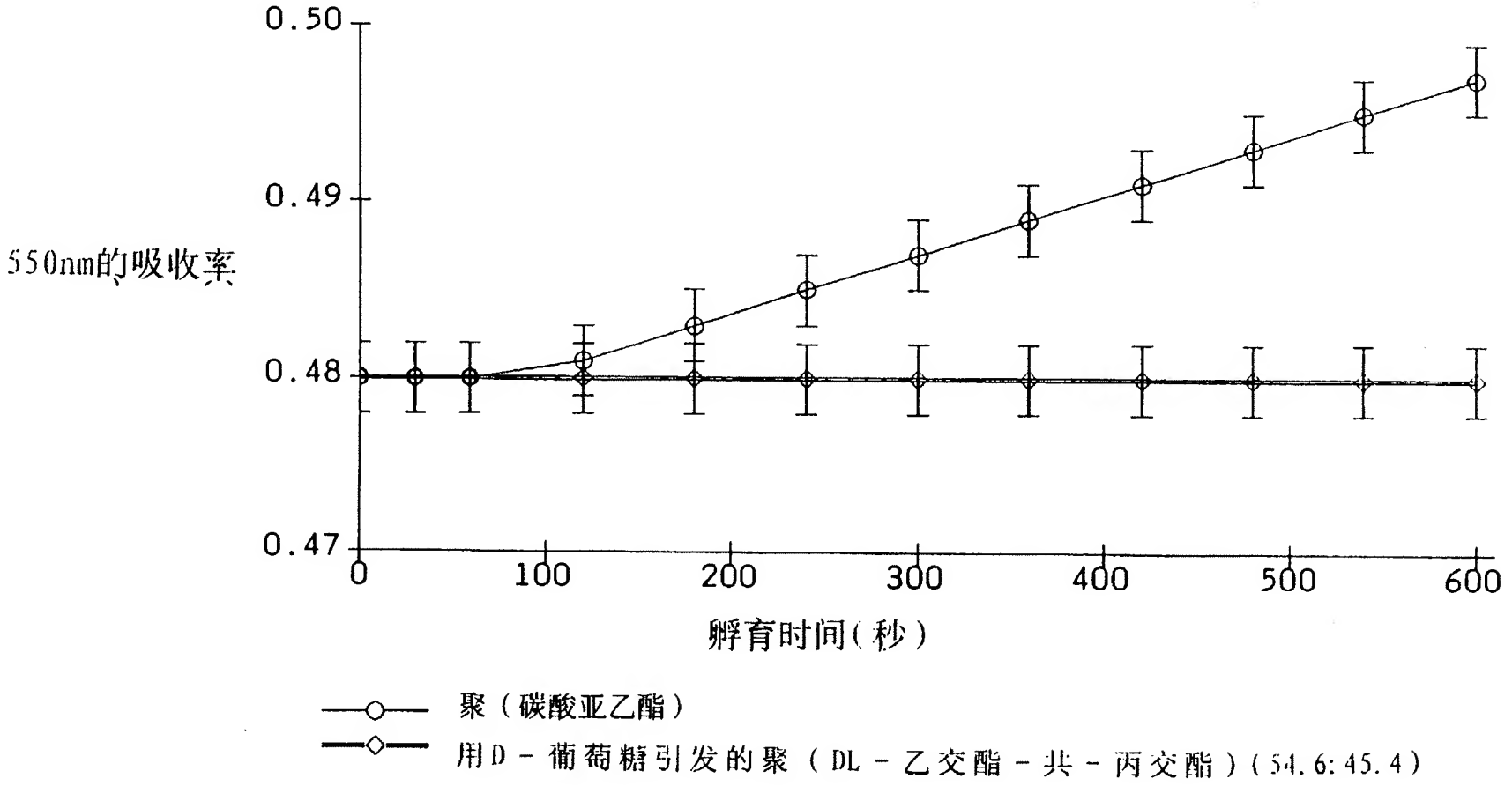


图6/13 聚（碳酸亚乙酯）体内和体外质降解的分子量函数

（仪器：扫描电子显微镜，n=2-6）

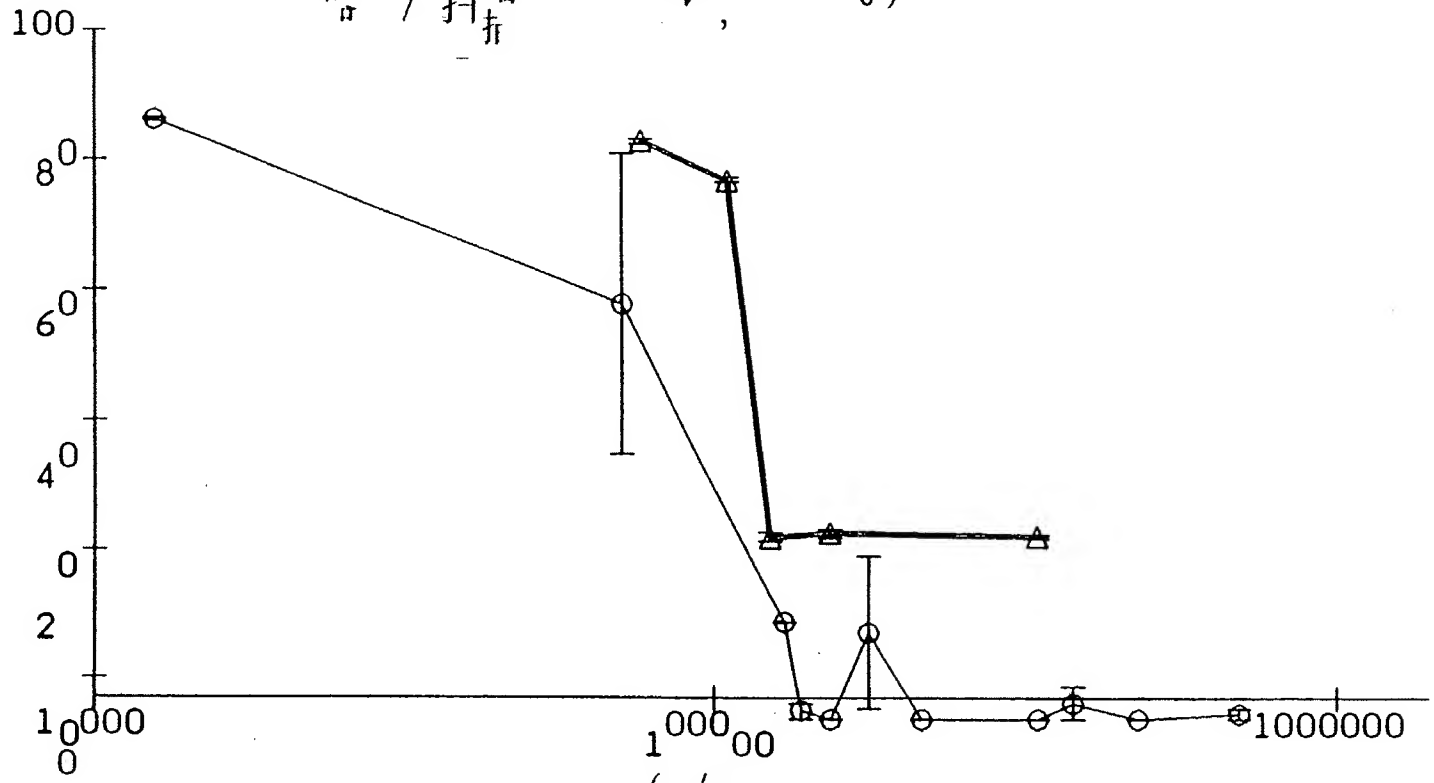


图7/13　鼠皮下用药14天后，过氧自由基清除剂对聚（碳酸亚乙酯）物质降解的影响（仪器 +/-扫描电子显微镜；n=3）

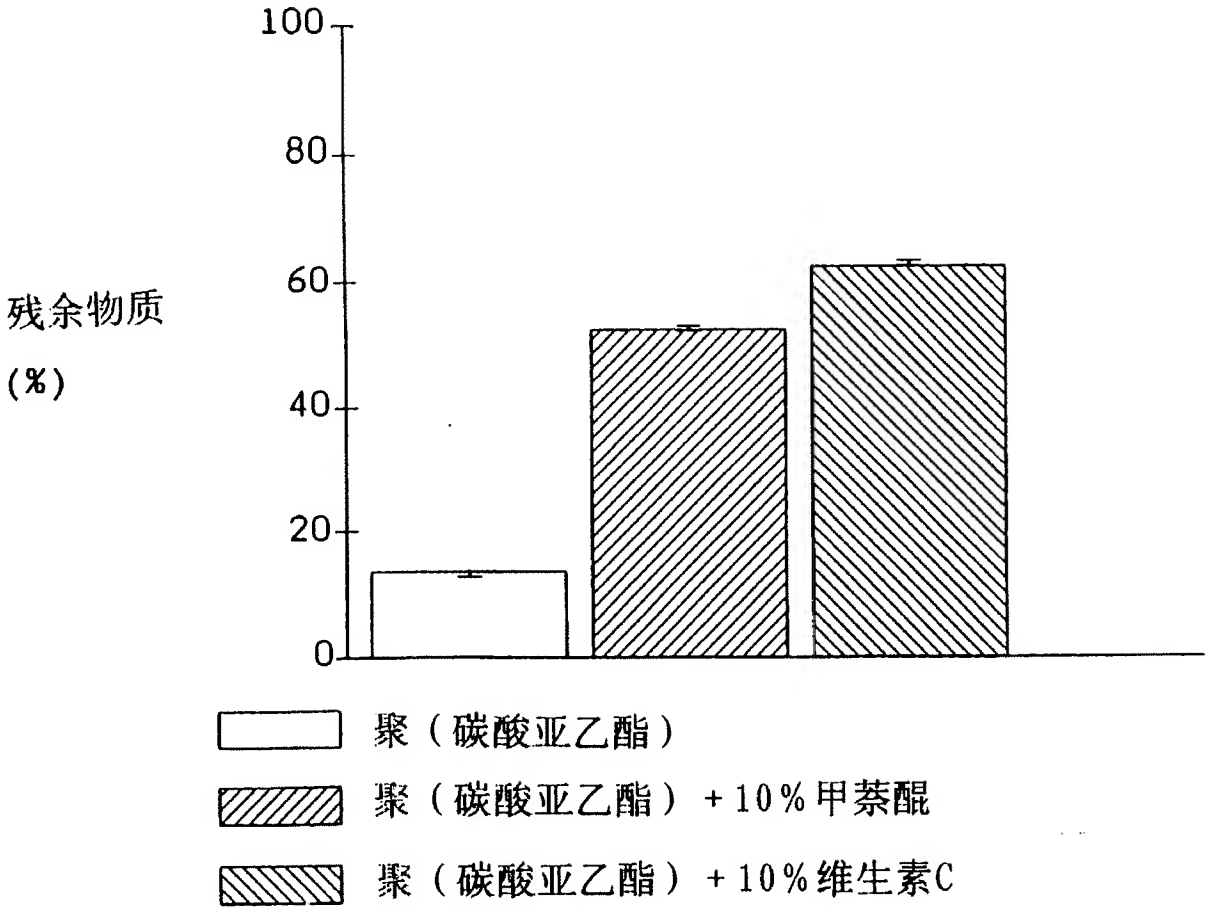


图 8/13 体内及体外聚（碳酸亚乙酯）的物质降解
（仪器 扫描电子显微镜，n=3）

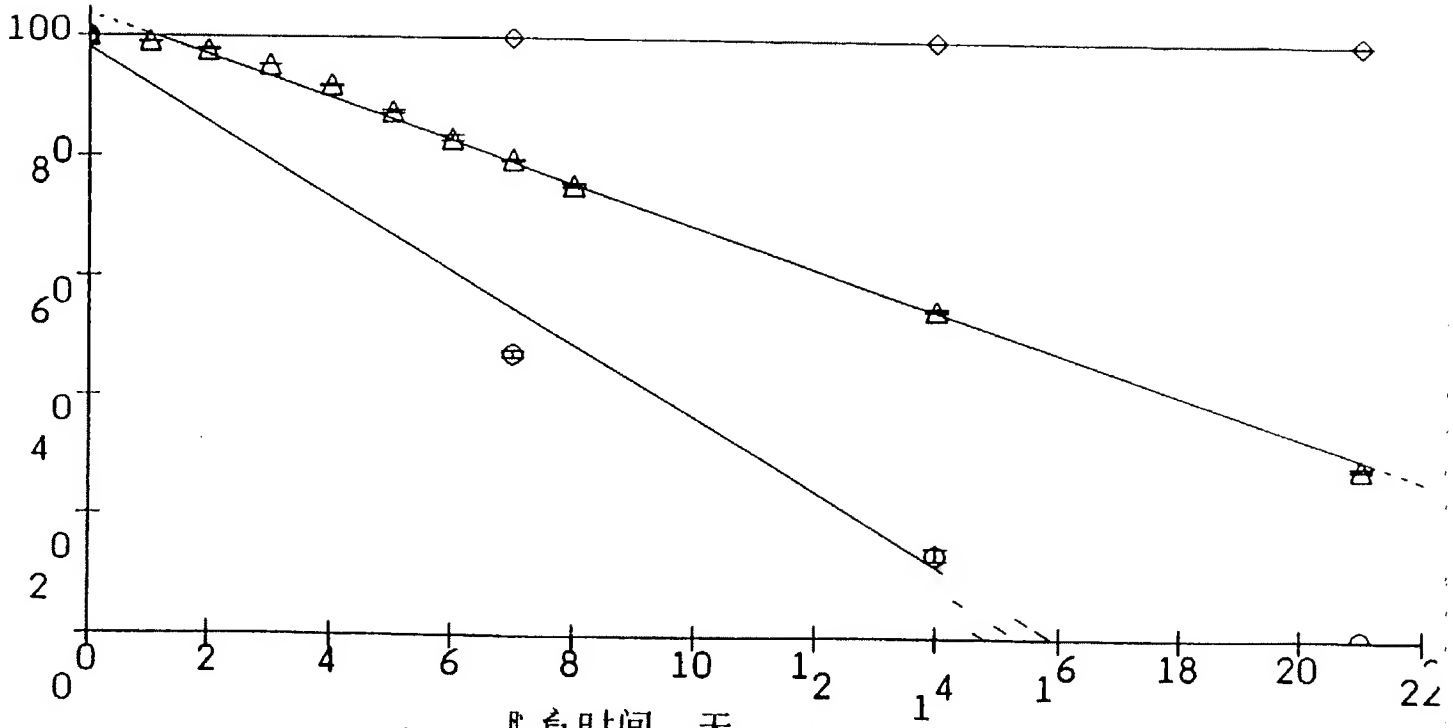
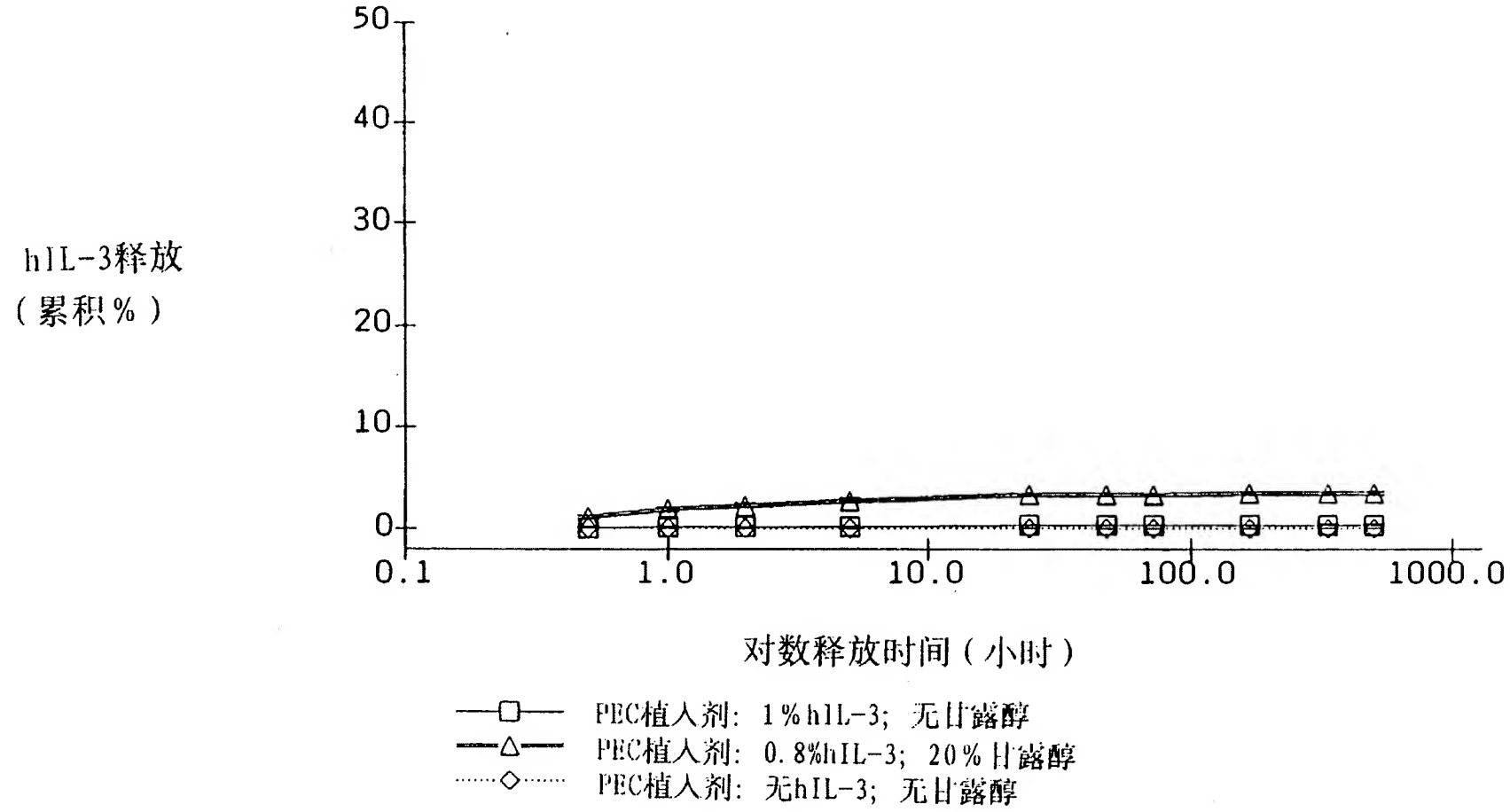


图9/13　体外从聚（碳酸亚乙酯）（PEC）植入剂中释放hIL-3（含血清培养基；hIL-3分析法：ELISA）（仪器 +/-扫描电子显微镜；n=3）



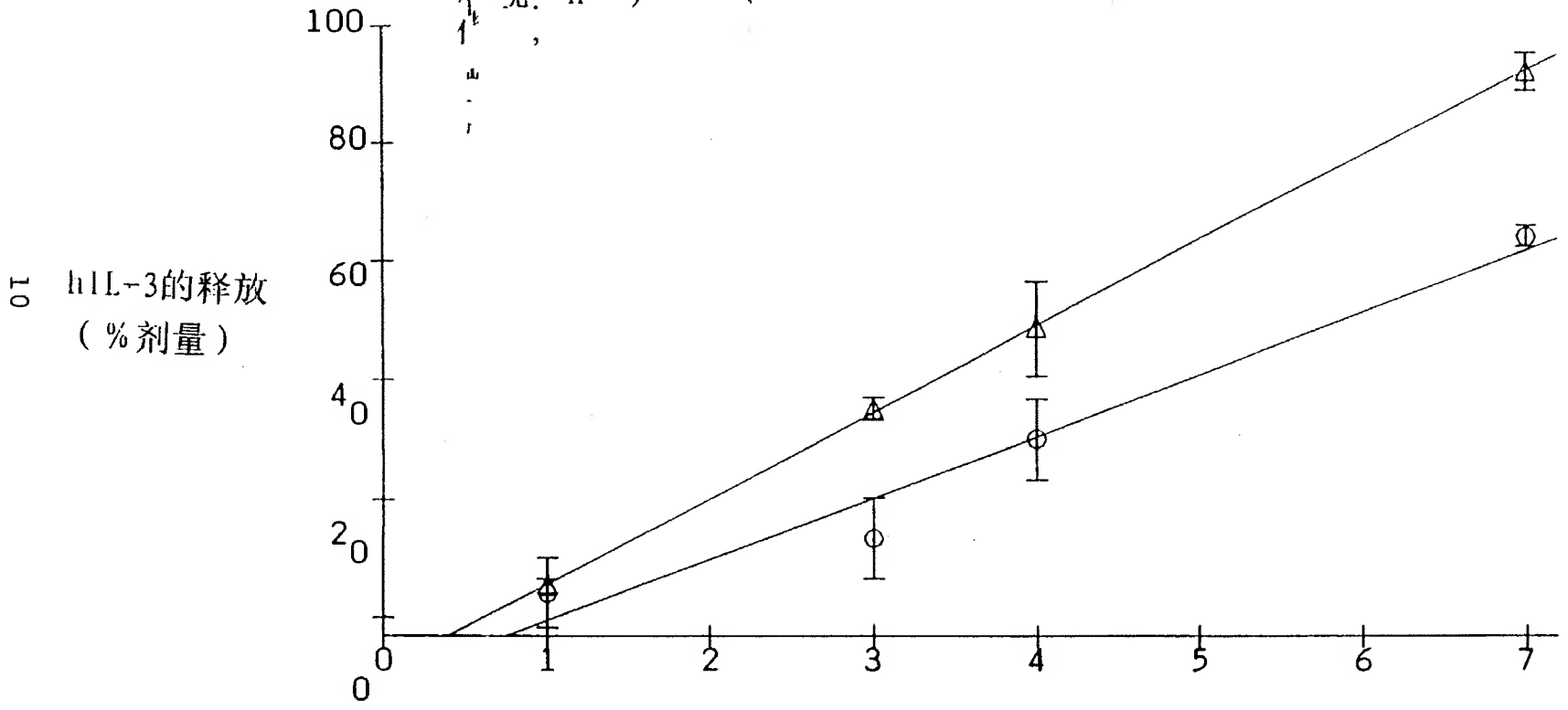
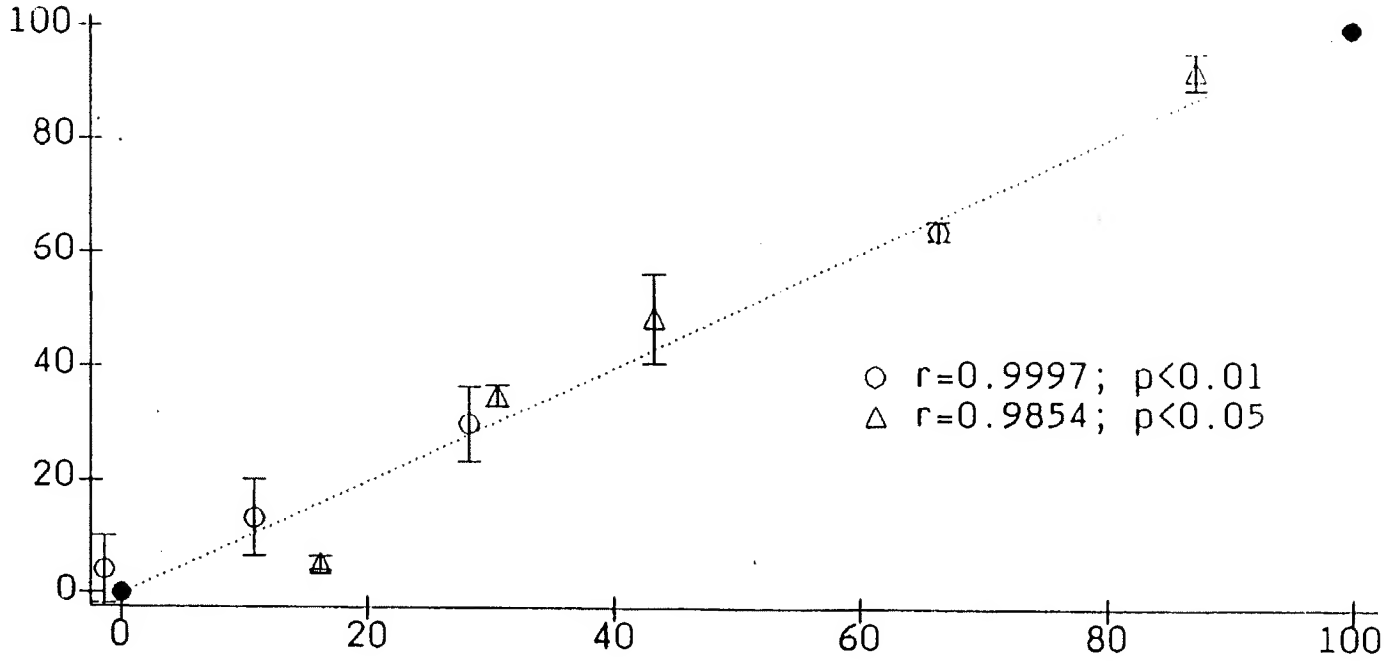
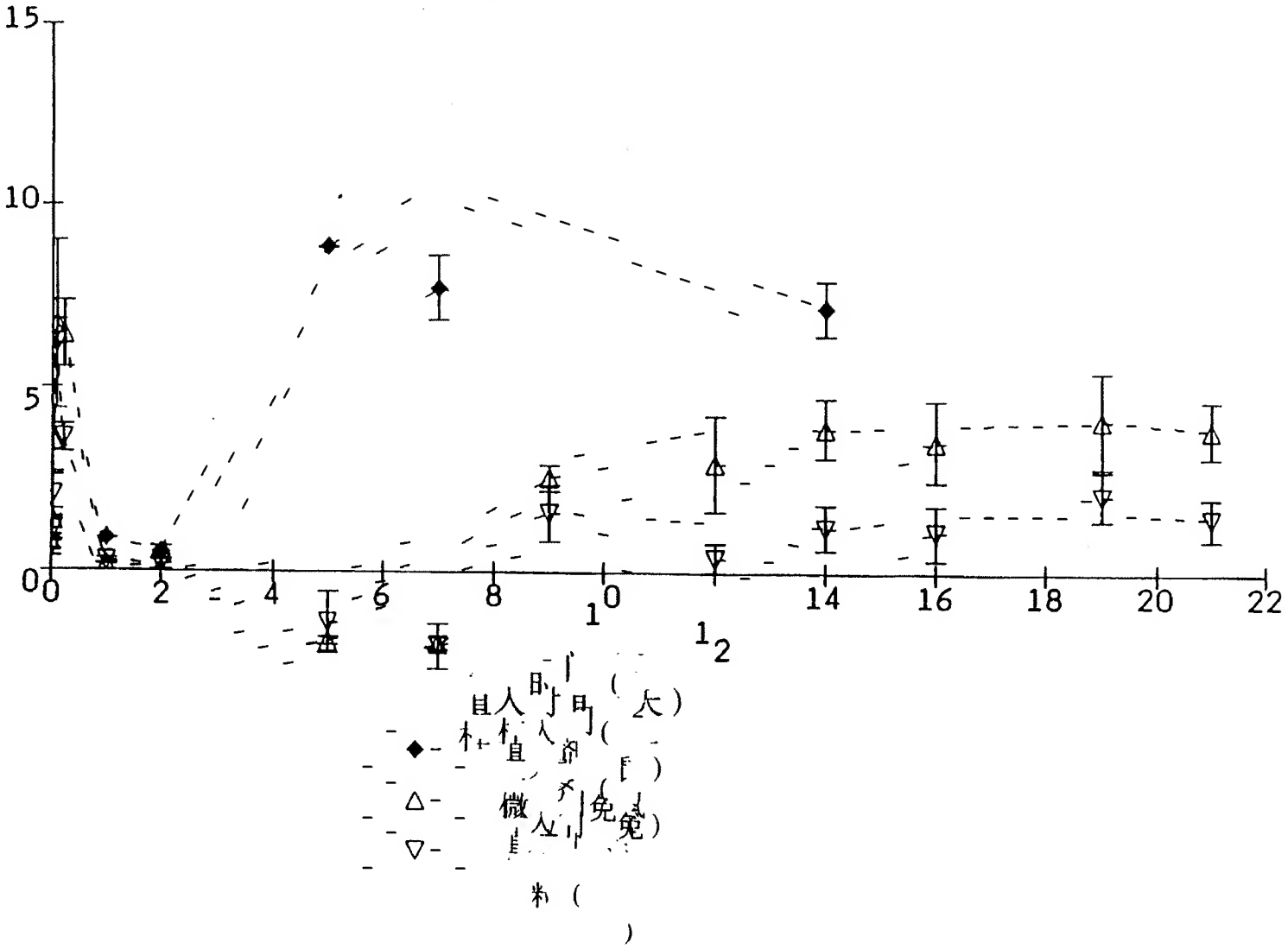

100
80
60
40
20
0
0
1
2
3
4
5
6
7
hIL-3的释放
（%剂量）
时间（天）

图11/13 鼠体内聚（碳酸亚乙酯）（PEC）物质损失与hIL-3释放的关系（hIL-3分析法：HPLC）（仪器 +/- 扫描电子显微镜；n=3）

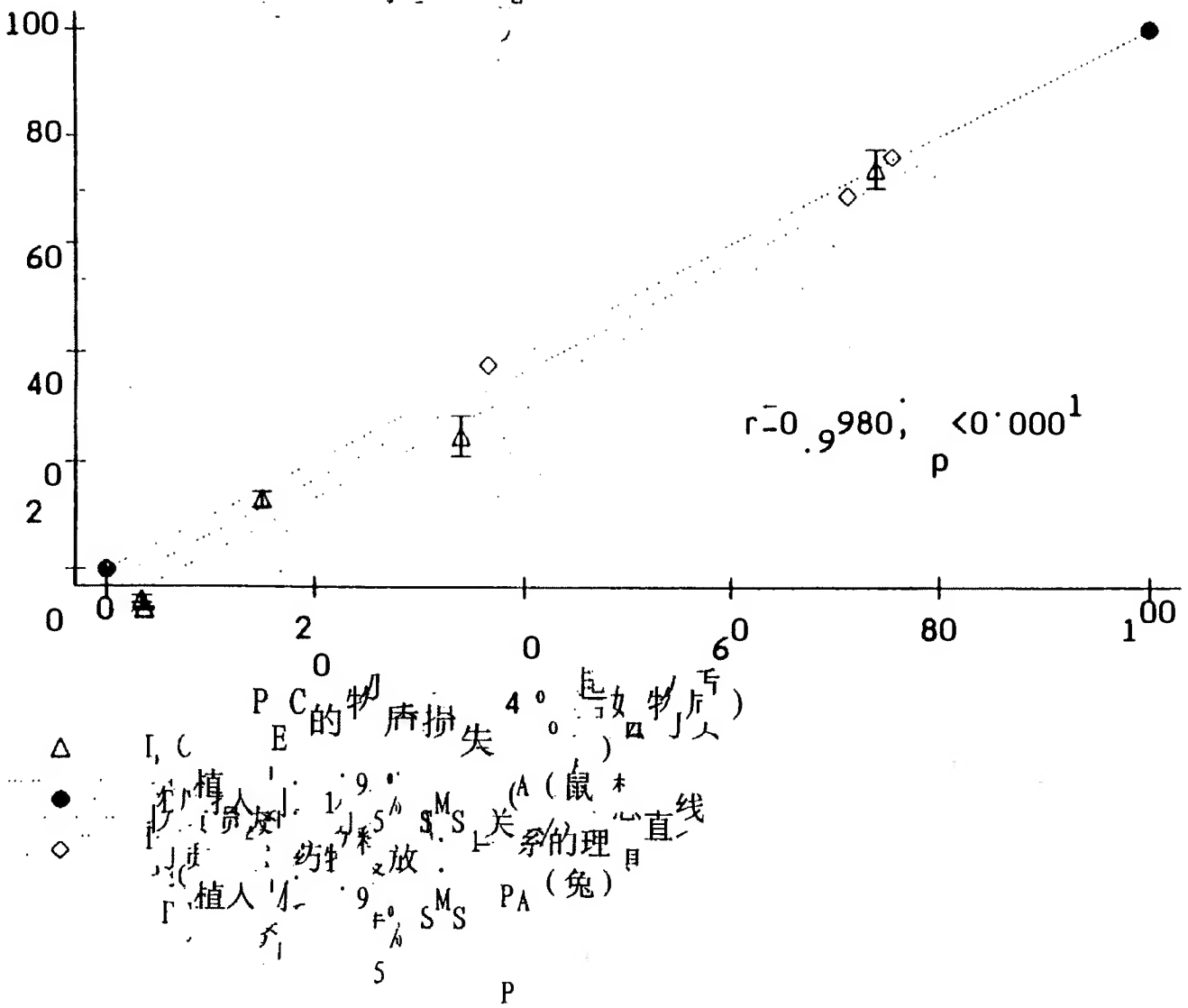


鼠及 SMS－PA血浆浓

仪 电



13/13 鼠及兔的聚（碳酸亚乙酯）（PEC）及SMS
的物质_失关系（皮下用SMS _PA，分析法：HPLC）
（仪器 +/- 电 ，n=2）



SMS-PA
释放
（%剂量）

13

P C的物质损失
△
●
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[54]发明名称 可生物降解的聚合物及含该聚合物的药物组合物

[57]摘要

本发明提供了可生物降解的聚合物和含有该聚合物的药物组 合物,所说聚合物含有式 A 的碳酸亚乙酯单元: $-(-C(O)-O-CH_2-CH_2-O-)-$ A 其中碳酸亚乙酯的含量为 70 至 100Mol%,在氯仿中在 20℃和浓度为 1g/dl 下测量特性粘度为 0.4 至 4.0dl/g,玻璃化温度为 15 至 50℃。

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权 利 要 求 书

1.一种可生物降解的聚合物，含有式A的碳酸亚乙酯单元:

-(-C(O)-O-CH_2-CH_2-O-)- A

其中碳酸亚乙酯的含量为70至100 Mol%，在20℃和浓度为1g/dl的氯仿中测量特性粘度为0.4至4.0 dl/g，玻璃化温度为15至50℃.

2.权利要求1的聚合物，以二氯甲烷为洗脱剂以聚苯乙烯为基准物通过凝胶渗透色谱检测其分子量(Mw)为100000至2000000.

3.权利要求1的聚合物，其碳酸亚乙酯的含量为90-100 Mol%.

4.权利要求1的聚合物，其比浓对数粘度在浓度为1 g/dl的氯仿中测定为0.4-3.0 dl/g.

5.权利要求1的聚合物，其玻璃化温度为18至50℃.

6.权利要求1的聚合物，它含有碳酸亚乙酯单元和环氧乙烷单元.

7.权利要求1的聚合物，它已接触沸腾的双蒸水5小时，经此处理后其玻璃化温度为18至50℃.

8.权利要求1-4或6中任一项的聚合物，其具有式B的环氧乙烷氧单元:

-(-CH_2-CH_2-O-)- B

作为共聚单元.

9.权利要求1-6任一项的聚合物，其含有羟基作为聚合物的一个末端基团.

10. 一种药物组合物，它包括权利要求1-9中任一项的聚合物.

11. 权利要求10的药物组合物，其中在聚合物中或上含有添加剂.

说 明 书

可生物降解的聚合物及含该聚合物的药物组合物

本发明涉及含有聚合物基质、特别是含有用于治疗 IL－1 和/或 THF α 介导的疾病（例如慢性炎性疾病）的 IL－6 的药物组合物。在此描述的本发明特殊聚合物（特别是聚（碳酸亚乙酯）聚合物），在含有药用活性化合物的缓释组合物中一般将它用作基质物质，特别是它在体内进行非水解表面溶蚀的性质是新的、出乎意料的，且特别符合要求。因此，含有其它药物的基质及聚合物的制备方法和含此聚合物的药物组合物也在此举例说明。此外，IL－6 治疗 IL－1 和/或 TNF α 介导的疾病的用途是新的并出乎意料（以前认为很多这样的疾病会因 IL－6 而恶化），于是本发明进一步提供了 IL－6 在治疗如慢性病原体诱发的炎性疾病、脱髓鞘疾病及急性和超急性炎性疾病（如败血症休克）中的新用途。

I. 治疗 IL－2 和/或 TNF α 介导的疾病

很多自发的慢性炎性疾病病因不明（可能是自身免疫）并被认为是由 IL－1 和/或 TNF α 介导的。例如，多发性硬化（MS）———一种特征为在脑和脊髓中存在脱髓鞘的播散斑的残缺神经紊乱，在很多年里成为研究单位的注意焦点。虽然多发性硬化的准确病因并不完全清楚，但根据征兆（如疾病患者的某种 HLA 抗原的频率增加）人们相信它存在强自身免疫因素。通常提供的抗炎药如 ACTH（促肾上腺皮质激素）或皮质甾类（如强的松）似乎会促进急性病的康复（特别是在早期使用），但不能治本。长期使用皮质甾类或免疫抑制剂会有严重的副作用。最近表明 IFN－β1 合剂降低短期的斑形成，但没有长期疗效。治疗效果的评估

很复杂，因事实上疾病的自然进程会自发地缓解并慢性复发．简言之，尽管广泛研究了很多年，但对这种严重的疾病还没有总体上可接受的特别治疗方法．

其它慢性炎性疾病被认为是由外源试剂（如病原体）诱导的．例如，莱姆病是由蜱生螺旋体疏螺旋体属 burgdorferi 感染引发的严重慢性疾病．起初急性期症状为皮肤损伤及流感样症状，之后疾病进人慢性期，它的症状为关节炎及慢性神经异常．通常用抗菌素和非甾类抗炎药治疗此疾病，但特别是对此疾病患者尚未发现适当的治疗方法．

急性或超急性不可控制的炎性疾病也可由内源性试剂引发（如严重的烧伤或严重的感染）．因目前尚无有效治疗方法，败血症休克是严重威胁生命的疾病．成人呼吸窘迫综合征（ ARDS ）也尤为如此．它的发病快，死亡率一般超过 50 %．败血症休克通常是严重细菌感染的结果，它的典型症状是发烧后期常伴有低温、血压波动（肌力过度症状）后期伴有低血压、代谢酸中毒、智力损伤及广泛的器官功能障碍，在很多病例中最后导致死亡．最常见的败血症休克是因革兰氏阴性细菌感染（内毒素），但也可因革兰氏阳性感染或其它感染．于是将在此使用的术语“败血症休克”解释成广义的由微生物感染（特别是细菌感染，最特别是革兰氏阴性细菌感染）引起的休克状态（包括 ARDS ）．

IL-6 是已知的细胞因子．可用于多种疾病的治疗，例如，血小板减少症及特定的癌症．它通常随机体对细菌感染的免疫而产生并且和炎症、发烧及败血症休克的调节有关．它是强免疫刺激剂，确有一些文献指出 IL － 6 作用机制引起某种自身免疫或炎性疾病，包括系统性红斑狼疮、多发性硬化、类风湿性关节炎及败血症休克．

因此，人们非常惊奇地发现 IL － 6 可用于治疗慢性炎性疾病（除肾

小球性肾炎外），如多发性硬化，及用于治疗急性和超急性炎性疾病，如败血症休克．其作用机理尚不清楚，但我们相信不用考虑任何特别的理论，只是通过反馈机理， IL - 6 可降低或抑制其它细胞因子的表达、释放或功能，特别是对 TNF α和/或 IL - 1 ，可能通过上升调节可溶性 TNF α受体和/或 IL - I 受体拮抗剂的释放，抑制其活性及其主要是这些细胞因子介导产生的自身免疫、炎症或休克症状．但在特征为 IL - 6 介导的补体激活抗原－抗体（ IgG ）复合物的疾病中，特别是肾小球性肾炎（它通常由这样的复合物在肾中沉积引起）， IL - 6 会加重病情．因此，我们强调 IL - 6 对动物模型的 MS 和莱姆氏关节炎（主要由 IL - I 和/或 TNF α造成）有治疗作用，但会加重狼疮鼠的肾小球性肾炎（直接由 IL - 6 造成）．我们也指明 IL - 6 自身对内毒素休克（同样假设其主要由 IL - I 和/或 TNF α造成）的鼠模型有治疗作用．

因此，认为特别是在治疗除肾小球性肾炎外的炎性疾病及治疗败血症休克中 IL - 6 可用作抑制 TNF α和/或 IL - I 的表达、释放或功能的试剂．可以用 IL - 6 治疗的炎性疾病包括如关节炎，特别是病原体诱发的关节炎如莱姆病关节炎、细菌诱发的关节炎及脊髓灰质炎性关节炎；多发性硬化及其他脱髓鞘疾病（即神经、大脑和/或脊髓脱髓鞘为特征的疾病，包括如多发性硬化、急性散布性脑脊髓炎或传染病后脑炎、眼神经脊髓炎、耳鸣、弥散性大脑硬化、席尔德病、肾上腺白质营养不良、第三期莱姆病、 tropical spastic parapoesis,及脱髓鞘特别是自身免疫介导的脱髓鞘为主要症状的其它疾病）；急性重症炎性疾病如烧伤、败血症休克、脑膜炎及肺炎；以及自身免疫疾病包括多软骨炎、硬皮病、韦格纳 granulamatosis 、皮肤肌炎、慢性活动性肝炎、重症肌无力、牛皮癣关节炎、史蒂文斯－约翰逊症、特发性口原性腹泻、自身免疫性

肠炎（包括如溃疡性结肠炎和节段性回肠炎）、内分泌眼病、凸眼性甲状腺肿、肉样瘤病、原发性胆汁型肝硬变、青少年糖尿病（Ⅰ型糖尿病）、眼色素层炎（前和后）、干性角膜结膜炎和春季角膜结膜炎以及间质性肺纤维化。

因此本发明提供了

i)抑制 TNF α和/或 IL－Ⅰ的表达、释放或功能的方法；

治疗或预防除肾小球肾炎外的炎性疾病的方法；

治疗或预防 TNF α和/或 IL－Ⅰ介导的疾病的方法；

治疗或预防上述任何疾病的方法；

治疗或预防脱髓鞘疾病如多发性硬化的方法；

治疗或预防外部诱发的炎性疾病的方法；

治疗或预防重症急性感染如败血症休克脑膜炎或肺炎的炎性反应；

治疗烧伤的方法；

治疗或预防病原体诱发的慢性炎性疾病如莱姆病的方法；

所述方法包括使用治疗或预防有效剂量的 IL－6，例如 TNF α和/或 IL－Ⅰ抑制量的 IL－6 如 rhIL－6（如特别是当 IL－6 作为单一的治疗或预防试剂使用时，或者与抗微生物或作用于血管的药剂任意联合使用，如任选不与 TNF α激动剂或拮抗剂或抗 TNF α抗体联合使用）；任选以缓释或贮存剂型如与聚合物基质（如下文描述的聚（碳酸亚乙酯）基质）联合用于需此治疗或预防的对象如哺乳动物、人；

ii)在制备用于方法（ｉ）如治疗或预防列于上述（ｉ）的任何一种疾病的药物中 IL－6 如 rhIL-6 的用途，其中药物是任意的缓释制剂如任选进一步含有聚合物基质（如下文描述的聚（碳酸亚乙酯）基质）；

iii)治疗或预防列于上述（ i ）中任何疾病中 IL - 6 如 rhIL - 6 的用途；及

iv)含有 IL - 6 如 rhIL - 6 的药物组合物，将其用于（ i)方法中，如治疗或预防上述（ i ）描述的任何疾病，任选以缓释制剂的形式，任选进一步含有聚合物基质（如下文描述的聚（碳酸亚乙酯）基质）；例如，在聚合物基质中含有 IL - 6 的缓释组合物（即在几天、几星期或几个月的时期内在体内生物降解的组合物），如以微粒或贮库的形式，其中聚合物在体内显示为非水解溶蚀，特别是本文描述的任何给药系统用于治疗上述任何疾病（如慢性炎性疾病）时.

IL - 6 指相应与白细胞介素 - 6（也称为β - 2 干扰素（ INF - β II ）、B 细胞因子 - 2 （ BSF - 2 ）、白细胞介素 - HP - 1 （ HR1 ）、肝细胞激活因子（ HSF ）、杂交瘤浆细胞瘤生长因子（ HPGF ）及 26kD 因子）的已知变体的任何化合物. 虽然非重组 IL - 6 也可使用，但优选重组 IL - 6（如由 IL - 6 分泌癌细胞系产生的）. IL - 6 可以购买或通过任何已知的方法制备（如 EPA0220574 、 EPA0257406 、 EPA0326120 、 WO88/00206 、 GB2063882 或 GB2217327 描述的，这些申请的内容在此引为参考）. 可将 IL - 6 糖基化（如由真核细胞如 CHO 细胞产生的）或非糖基化（如由原核细胞如 E， Coli. 产生）. 虽然已知 IL - 6 为活性杂交种，但优选重组人 IL - 6 （ rhIL - 6 ），也可使用来自非人源的 IL - 6 并将其包括在本文 IL - 6 的含义范围内. 在 IL - 6 的序列中具有微小变化（如增加、删除或诱变 1 、 2 、 3 或多个氨基酸）的蛋白质、含有 IL - 6 及其它蛋白的融合蛋白质、 IL - 6 的活性片断和/或其它具有 IL - 6 活性的 IL - 6 的变体、截断的或突变的形式包括在本文 IL - 6 的含义范围内.

含有IL－6和药用稀释剂或载体的适宜的药用组合物是已知的．IL－6可非肠道给药，如以注射溶液或混悬液的形式，按照或类似于Remington's Pharmaceutical Science,第16版（Mack Publishing Company,Easton,PA 1980）的描述．适宜的载体包括水载体如生理盐水、Ringer氏溶液、右旋糖溶液及Hank氏溶液以及非水载体如脂肪油和油酸乙酯．对于一般的非肠道给药，单位剂量的IL－6以冻干的形式，它可与载体混合形成适当的注射用溶液或混悬液．

另外，IL－6可用植入或缓释药物给药系统，如微粒或贮库制剂与聚合物一起形成聚合物基质，而药物从基质中缓慢释放．在所治疗的疾病为慢性病（如慢性炎性疾病）且所需治疗持续若干星期或若干月时优选这种方式．聚合物是指由重复单元任何适当（如可药用）线性高分子量分子（包括均聚物、共聚物及杂聚物）组成，其可是任意的支链或胶联的形式，如通过一种分子聚合或多于一种分子共聚（如由下文描述的环氧乙烷和二氧化碳形成的聚（碳酸亚乙酯）），并且可在聚合物链上任意含有其它单元的嵌段．优选的聚合物为线性且由碳、氧和氢组成，如聚－DL－丙交酯－乙交酯共聚物、聚乙二醇或聚（碳酸亚乙酯）．优选的聚合物表现出非水解溶蚀，如本文进一步描述的聚（碳酸亚乙酯）．

所用的剂量当然随所用IL－6的准确类型、宿主、给药方式及治疗病症的特点和严重程度变化．通过皮下注射或缓释剂型给较大的哺乳动物用药的每日剂量为0.5μg/kg至30μg/kg，优选2.5μg/kg至10μg/kg，或以IL－6的其它任何安全及有效的体内活性剂量用于治疗，如以血小板增加的剂量．在重症急性炎性疾病如败血症休克中，需要较高剂量静脉给药以达到快速和较强的反应．IL－6给药的频率可由每日给药降至隔日或每星期或在使用缓释剂型时间隔更长的时间给药（这在

长期治疗时优选）。IL－6治疗可导致寒战、发烧及流感症状，这一般可共同使用非麻醉止痛药如阿斯匹林、扑热息痛或消炎痛治疗或预防。其它显著的副作用一般仅在高剂量如高于每日10μg/kg时出现，并一般可通过降低剂量缓解。

II.缓释用聚合物基质

本发明进一步提供适于药物缓释的药物组合物，它适于在上述征兆时IL－6及其它药物的给药。此药物组合物特指那些含有聚（碳酸亚乙酯）聚合物，有时称作聚（碳酸亚乙酯）类或PEC类。

虽然现有技术提供了一些聚（碳酸亚乙酯）类用于药物给药系统的实例，但现有技术并未公开本发明的特定聚合物，同时也未公开此聚合物能在体内进行非水解溶蚀。现有技术也未公开本文公开的特定药物（如IL－6）的给药系统，也未提出这样的药物需缓释系统。

特别令人惊奇的是本发明聚合物的生物降解特性。根据一般化学基础知识，预计碳酸酯键为主要的裂解键。然而，聚碳酸酯在体外适当的条件下是稳定的。

按照Chem. Pharm. Bull. 31(4),1400-1403(1983)，聚（碳酸亚乙酯）类可在体内进行生物降解，但所试的聚合物不能通过如现代光谱法明确地鉴定。按照1402页，体内生物降解只能解释为由于水解酶的作用。

按照Chem. Pharm. Bull. 32(7),2795-2802(1984)，由含地布卡因的聚（碳酸亚乙酯）制成微粒。虽然该描述涉及非常相关的技术，但从中并未看出地布卡因的释放与聚合物的体外或体内生物降解有关。同样所试聚（碳酸亚乙酯）的物理及化学性质未得到充分说明。

按照Makromol. Chem. 183, 2085-2092(1982)（特别是2086页），

二氧化碳环氧乙烷聚合物可生物降解，它还指出初步的结果证实了二氧化碳－环氧乙烷聚合物的生物降解性及其由此带来的它们在控制药物释放中的应用．为支持生物降解性的断言它引用了 Jinko Zoki 3(Suppl.),212(1974)．此出版物说明聚（碳酸亚乙酯）属最易水解的一组化合物，甚至链霉蛋白酶在分解它时也毫无困难．既然链霉蛋白酶由水解酶混合物组成，这意味着在体外及体内酶水解是可能的．但这个结论非常令人怀疑．我们将本发明的聚（碳酸亚乙酯）类以直径 5mm 、重量 25mg 的压片形式加入到 10mg/ml 链霉蛋白酶和 5mM $CaCl_2 \cdot 2H_2O$ 的 pH 7.4 的磷酸盐缓冲生理盐水（ PBS)液中及 10mg/ml 链霉蛋白酶 E 和 5mM $CaCl_2 \cdot 2H_2O$ 的 pH 7.4 的磷酸盐缓冲生理盐水（ PBS ）液中（在 37 ℃）没有观察到生物降解（见图 1 ）．链霉蛋白酶溶液每天更新．

现在惊奇地发现选择具有特殊碳酸亚乙酯的含量、粘度及玻璃化温度范围的不能水解生物降解（如在水解酶如链霉蛋白酶的存在下或在碱性条件下）的聚（碳酸亚乙酯）类在体外和体内不再生物降解，即只是通过表面溶蚀．“表面溶蚀”的表达方式用于本发明，特别与聚酸酐及聚原酸酯的水解生物降解有关，但决不仅仅限定于此．

如果物质的生物降解仅仅在聚合物颗粒的表面，而其余聚合物残留物的分子没有降低，表面溶蚀便发生了．在文献中声明观察到的表面溶蚀中，没有进行符合物质损失检测的残余物分子量测定，因此实际上从未证实过表面溶蚀．

实际上，对至今几乎所有所试的聚合物，只观察到聚合物整体溶蚀．具有聚合物整体溶蚀的系统有显著的缺点，即如果聚合物载有药物化合物如肽，肽可能释放到生物介质中，在生物介质的影响下相当不稳定．药物化合物的主体部分已与介质接触，早在其从聚合物中释放前便可能

失去活性．如果聚合物能进行表面溶蚀，即当没有整体溶蚀发生时，植入的药物化合物（如肽）可在表面溶蚀达到药物颗粒及药物颗粒从残余聚合物的表面释放前得到保护，不受生物介质的有害影响．在聚合物基质药物系统中，显示有表面溶蚀而不是整体溶蚀，药物颗粒接触生物介质的有害影响的时间较短，由此能使药物活性物质从聚合物基质中的释放时间较长、量较高并且较稳定．

在最近的出版物 Proc. Nat. Acad. Sci. USA 90552-556(1993) 和 904176 - 4180 (1993)中描述了对聚酸酐的类似表面溶蚀的一些特性．但是仍受整体溶蚀的影响并且没有进行分子量测定．此外这种溶蚀是水解类型的．现在发现选择聚（碳酸亚乙酯）类的组份（在下文定义）表明在体外及体内绝对都是非水解表面溶蚀．

本发明提供了在体内及体外通过由非水解机理控制的表面溶蚀降解的聚合物，它具有式 A 的碳酸亚乙酯单元:

$$-(-C(O)-O-CH_2-CH_2-O-)- \qquad A$$

其中碳酸亚乙酯的含量为 70 至 100 Mol %，在氯仿中在 20 ℃测量特性粘度为 0.4 至 4.0 dl/g，玻璃化温度为 15 至 50 ℃．

本发明的聚合物的碳酸亚乙酯的含量为 70 至 100Mol %，特别是 80 - 100 %，优选 90-99.9%，如 94-99.9%．聚合物的特性粘度在氯仿中在 20 ℃测量为 0.4 至 4.0 dl/g．优选聚合物具有比浓对数粘度（在 20 ℃及粘度为 1g/dl 的氯仿中测量）为 0.4 至 3.0 dl/g．

玻璃化温度为 15 至 50 ℃，优选 18 至 50 ℃．

在文献中已描述了玻璃化温度为 5 至 17 ℃的聚（碳酸亚乙酯）类．

本发明的聚合物优选由环氧乙烷和二氧化碳共聚制备，其制备方法也是本发明的一部分．作为本制备方法的结果，在大多数实例中聚合物

含有式 B 的环氧乙烷单元作为共聚单元

$-(-CH_2-CH_2-O-)-$ B

如果本发明的聚合物接触水介质（如 pH7.4 磷酸盐水缓冲液），实践证明没有介质迁移到它们的主体部分（见图 2）。因此在至少 28 天内未发生整体溶蚀并且残余物保持常数（100 %），见图 3 中的右图。

目前聚－ DL －乙交酯－丙交酯共聚物是最常用的缓释药物系统的基质物质。但这样的聚合物与本发明的聚合物不同，通过水解降解。例如，在 PBS 中的物质降解显示于图 3 的左图，这是最复杂的聚－ DL －乙交酯－丙交酯共聚物类型之一，即葡萄糖引发的聚－ DL －乙交酯－丙交酯共聚物（ DL － PLGGLU ），描述于英国专利 GB2145422 。

本发明的聚（碳酸亚乙酯）类与现有技术的聚－ DL －乙交酯－丙交酯共聚物之间的体内降解行为的不同也如图 3 所示。其中聚乙交酯－丙交酯共聚物进行整体溶蚀，正如所见的 DL － PLGGLU 的残余物的分子量降低，而聚（碳酸亚乙酯）类的残余物的分子量保持常数（ 100 %）。

在一个月内两个例子中体内的总植入物的残余物降至零，它意味着聚（碳酸亚乙酯）进行表面溶蚀而不是整体溶蚀。作为缺乏整体溶蚀的结果，在贮存期间（即在给药前）载体聚合物不让潮气渗入，保持与制备时相同的干燥状态。如果其中包藏的药物对潮湿敏感则也会保持稳定。

本发明也提供了制备聚合物的方法，其中环氧乙烷和二氧化碳以 1： 4 至 1： 5 的摩尔比在催化剂的作用下聚合。如果两个环氧化合物的分子互相反应而没有二氧化碳分子的干扰，即如果含氧阴离子中间体在被二氧化碳羧基化前进攻另一个环氧乙烷分子，则很清楚在本反应的范围内在聚合物链上引入环氧乙烷单元是可能的。这样聚合物可能含有

若干个环氧乙烷单元．本发明的聚合物如果含有环氧乙烷单元则具有碳酸亚乙酯和环氧乙烷单元的随机分布，表示为总式 Am-Bn=

$$-(C(O)-O-CH_2-CH_2-O-)_m-(-CH_2-CH_2-O-)_n$$

其中

$$\frac{m}{n+m} \times 100 = 70 \text{ 至 } 100$$

但是本发明聚合物中的多数环氧乙烷单元据统计具有邻近的碳酸亚乙酯单元，特别是在环氧乙烷单元的摩尔比例小的实例中．这意味着在这些实例中所得的多数醚官能团随机分布在聚合物链的碳酸官能团之间．在 CDC13 中本发明产品的 1H - NMR 证明了这种假设．它们在 δ = 约 4.37ppm(全部 Ia ）显示了碳酸亚乙酯单元（在两个碳酸官能团之间的乙烯单元）、在约 4.29 和 3.73ppm （全部 Ib 和 Ic ）在一个碳酸和一个醚官能团之间的乙烯单元及在约 3.65ppm （全部 Id ）在两个醚官能团之间的乙烯单元的信号．碳酸亚乙酯单元的比例（ A ）就可在 NMR 精确度内按下式计算:

碳酸亚乙酯单元的 Mol%（ A ）：

$$\frac{Ia}{Ia+Ib+Ic+Id} \cdot 100$$

作为聚（碳酸亚乙酯）类的结构特征，在文献中常给出它们的醚官能团的含量，而不是其碳酸亚乙酯的含量．本发明的聚合物中醚官能团的比例（ E ）可按下式计算:

醚官能团的 Mol%(E)=

$$\frac{Ic+Id}{Ia+Ib+Ic+Id} \cdot 100$$

按照 PCT 专利申请 WO92/22600 制备聚（碳酸亚乙酯）类，其中以 2 至 400 : 2 的摩尔比含环氧乙烷单元和碳酸亚乙酯单元，这意味着聚合物含有至少 50Mol %的环氧乙烷和少于 50Mol %的碳酸亚乙酯单元．此申

请提到聚合物的生物降解性和它们作为生物可溶蚀基质用于缓释药物活性化合物的用途．但是没有给出数据证实聚合物确实能生物降解．总之，具有如此大量的醚官能团的聚（碳酸亚乙酯）类很少能生物降解．此申请未提及任何有关此聚合物表面溶蚀可能性的暗示．

美国专利 3248415 的实施例中描述了含有少于 70Mol %的碳酸亚乙酯单元的低分子量聚（碳酸亚乙酯）类（ Mw = 700 - 5000 ），这不同于本发明的聚合物并且未提及任何有关的生物降解性．

PCT 专利申请 WO89/05664 描述的聚（碳酸亚乙酯）类含有结构 II 环氧乙烷和碳酸亚乙酯单元的摩尔比为 1 至 8： 1，这意味着聚合物含有至少 50Mol %的环氧乙烷和最多 50Mol %的碳酸亚乙酯单元，这不同于本发明的聚合物．虽然描述了此聚合物用于可生物降解的给药器如含有药物化合物的植入物，但没有给出有关表面溶蚀的信息．

本发明的方法中，环氧乙烷单元的含量和醚官能团的含量延缓或抑制了聚合物的生物降解速度，通过选择反应条件如反应组份的摩尔比率、反应温度并进一步选择合适的催化剂降低其含量，催化剂可由二乙基锌和水或丙酮或二－或三酚（如间苯三酚）分别以摩尔比 0.9:1 至 1:0.9 或 2： 1 至 1： 2 制备，或优选由二乙基锌和二醇(特别是乙二醇)以 0.9:1 至 1:0.9 的摩尔比制备．

此方法优选在有机溶剂（如二噁烷和二氧化碳）的溶剂或分散剂系统进行．优选二氧化碳以液体形式使用并过量存在．压力优选 20 至 70 巴并优选温度为 10 至 80 ℃，特别是 20 至 70 ℃．

这样制得的本发明聚合物通常含有少于 15 %的醚官能团，优选少于 10 %，特别是少于 5 %（如少于 3 %）．本发明的聚（碳酸亚乙酯）类如果使用由甘醇或丙酮和二乙基锌制备的催化剂制备则其表现有低多分

散性（ Mw/Mn ），通常小于 5，如小于 2.5．

本发明的方法中催化剂或部分催化剂被看作是（共）聚合物的链引发剂．当反应完成且链生成时，其末端基团是羟基．链的相反位点（链的起始点）可被催化剂基团或其片段占据．如果催化剂由乙二醇和二乙基锌或水和二乙基锌制备，则聚合物链的两端相同．但是如果催化剂是由二－或三酚和二乙基锌制备的，芳族基团将被引入链的末端（这是链的起始点），而链的另一端为羟基．由图 4 可见，如果聚（碳酸亚乙酯）的一个末端基团被封端，例如，被如间苯三酚的芳香引发剂封端，则它的生物降解较慢．因此人们假设聚合物链降解起始于一个羟基末端或多个羟基末端．或者，也可考虑末端羟基的随后衍生化（如通过酯化）封端羟基并控制本发明的聚（碳酸亚乙酯）类的生物降解．适宜的末端酯基团为生物相合的酯基团，如（ C_{1-48} ）脂肪酸酯基团，优选（ C_{1-30} ）特别是（ C_{1-18} ）的脂肪酸酯基团，例如乙酸和硬脂酸的基团，或碳酸酯基团如碳酸亚乙酯基团、或双羟萘酸酯基团或乳酸、或乙醇酸、或聚乳酸、或聚乙醇酸、或聚乳酸－共－乙酸酯基团．

本发明的聚（碳酸亚乙酯）类在热水（ 90 － 100 ℃）中可稳定数小时，而没有明显的分子量降低．在接触沸腾的双蒸水 5 小时后观察到玻璃化温度显著增加，如达到高于 18 ℃（如 28 ℃）．通过这个反应步骤，得到较高的聚合物纯度．我们发现用此方式处理聚合物也较容易．

如前所述，本发明聚合物的聚（碳酸亚乙酯）部分是不可水解的，即在生理条件下通过水解酶或在 pH12 及 37 ℃至少一个月内不水解（见图 1 和 8 ）．但是发现本发明的聚合物在体内和体外在过氧自由基阴离子 O_2^- 的作用下通过表面溶蚀降解．过氧自由基阴离子 O_2^- 在体内和体外的炎性细胞中在本发明聚（碳酸亚乙酯）类存在下产生（见图 5 ）．聚

乙交酯－丙交酯共聚物目前最常用作药物缓释系统的基质物质，通过整体水解降解，不能诱发过氧自由基阴离子O_2^-的产生，在同一图中显示了葡萄糖引发的聚－DL－乙交酯－丙交酯共聚物，也可见图3。

在体外建立了含有过氧化钾的水溶液体系作为O_2^-的来源，并显示了本发明的聚（碳酸亚乙酯）类的表面溶蚀（见图8）。在体外选择pH12，因为O_2^-自由基在此pH值充分稳定。

有趣的是，不同于乙烯单元的氢被甲基取代的聚（碳酸亚乙酯），聚（碳酸亚乙酯）几乎不可生物降解，见 Chem. Pharm. Bull 31(4), 1400-1403(1983)。

使用本发明的聚（碳酸亚乙酯）类的微粒混悬液在48只鼠和24只狗分别进行了21天和35天的毒理研究。每种在第1天和第17天使用两次。经皮下及肌肉使用聚合物微粒10和40mg/kg体重后，未观察到系统临床毒性，未观察到对血液学参数、临床血液化学参数、体重和进食量的相关影响。在给鼠用药4和21天及给狗用药18和35天后测定用药位置的组织病理学变化。除了预期的炎性反应外，未发现不正常的组织病理学变化。

本发明聚合物的降解速率可在大范围内调整，这依靠于它们的分子量、环氧乙烷的含量、末端基团的性质（如生物相合的酯基团）、及O_2^-自由基清除剂（如维生素C）的存在，并可持续5天至6个月或更长，如高达1年。自由基清除剂优选作为添加剂置于聚合物中。

本发明的（共）聚合物的分子量Mw为80000（优选100000，特别优选200000）至2000000道尔顿，这可用二氯甲烷作为洗脱剂以聚苯乙烯作为参照物通过凝胶渗透色谱测定。

上面讨论的 Chem. Pharm. Bull. 32(7) 2795-2802(1984)提到使用

了分子量为 50000 至 150000 道尔顿的聚（碳酸亚乙酯）类．我们发现聚合物在体内和体外降解只有在分子量高于 80000（优选 100000）（图 6）时才能令人满意．这也正是本发明的内容．

本发明的聚合物可用于药物组合物，特别是作为包藏药物活性化合物的基质物质．因为在体外和体内条件下未发生整体溶蚀并且活性化合物被聚合物保护，故由于基质的表面溶蚀活性化合物一出现在基质表面（而不是在这之前）就立即释放．在体外 pH7.4 不含 O_2^- 的水溶液系统中，只有痕量的活性化合物释放（见图 9）．

表面溶蚀的另外一个优点是药物活性化合物分子的大小不影响释放的速率．

于是本发明提供了一种存在于聚合物中的药物活性化合物的药物组合物，此聚合物表现出非水解表面溶蚀，特别的活性化合物的释放与非水解聚合物的物质降解是直线关系（特别是 1：1 的直线关系）且活性化合物在聚合物基质中得到保护．

组合物优选以微粒或植入片的形式使用．

可通过已知的方法制备本发明药物的制剂，通过适当的喷雾干燥或乳化技术制备微粒，通过将药物化合物和聚（碳酸亚乙酯）类的固体颗粒混合，在高温下聚（碳酸亚乙酯）类软化而便于处理，随后任意将混合物冷却成固体并制成适宜的形状．也可将溶解或分散状态的药物化合物与聚（碳酸亚乙酯）溶液混合并蒸发溶剂，此后将固体残余物制成适当的植入片剂型．

含有微粒的药物组合物可通过将其与适当的草本制剂赋形剂加工处理并将其任意置于适当的分散剂中制备．

根据药物性质及制备方法，药物的含量可在大范围内变化，其重量

比由 0.001 至约 70 %，如 0.001 至 20 %，优选 0.001 至 5 %．应避免药物含量高导致介质渗透入聚合物中，这限制了加药量的上限．

在使用药物化合物的医学实践中，药物活性化合物的每种类型都可用于与本发明的聚（碳酸亚乙酯）合用．对于微粒优选的药物化合物类型是在低含量具有药物活性并在长时期内其血药浓度需恒定，如激素、肽或蛋白质（如生长激素释放抑制因子、干扰素或细胞介素），但特别优选那些不稳定并且口服后在胃肠系统会分解并因此非肠道给药的药物．

本发明的贮库剂型可用于多类活性制剂的给药，药物活性制剂如避孕药、镇静药、甾类、磺胺类、疫苗、维生素、抗周期性偏头药、酶、支气管扩张剂、心血管药物、止痛药、抗菌素、抗原、抗痉挛药、抗炎药、抗帕金森药、催乳激素抑制剂、抗哮喘药、老年病用药及抗肿瘤药．活性制剂可在广泛的化学化合物中挑选，如亲脂性或亲水性活性制剂，包括肽如 octreotide（描述于英国专利 GB 2234896 A）．

活性蛋白质或肽优选细胞因子，如白细胞介素、G - CSF、M - CSF、GM - CSF 或 LIF、干扰素、红细胞生成素、环孢菌素或激素，或它们的类似物（如 octretide）．

药物组合物可用于:

免疫调节，其中活性成分包括细胞因子如白细胞介素（IL - 3、IL - 6）或成血集落刺激因子（G - CSF 如 Filgrastim、GM - CSF 如 Molgramostim、Sargramostim、M - CSF），如作为疫苗的辅剂;

在骨髓抑制治疗或骨髓移植后重新造血的目的，活性成分包括成血生长因子如 GM - CSF、G - CSF、IL - 3、IL - 6、白血病抑制因子（LIF）、干细胞因子（SCF）或其合剂;

使活性成分的局部浓度高，其中活性成分含有药物或细胞因子、GM－CSF、IL－6、IL－2、IL－4或其合剂，当与辐射后肿瘤细胞或疫苗抗原（类似于用相应细胞因子基因转染的辐射后肿瘤细胞）一起用药时刺激保护性免疫反应；

诱发强免疫反应，其中活性成分包括如GM－CSF与抗原联合用药；特别是与肿瘤抗原、病毒抗原或细菌抗原；

局部注射组合物使伤口愈合，如其中活性成分包括GM－CSF；

引发抗原特异免疫的耐受性，其中活性成分为如GM－CSF与辅助分子（助受体）的抑制剂合用，CD28－B7相互作用、CD40－CD40配体相互作用、粘联因子相互作用的特别抑制剂；

与抑制细胞生长治疗联合治疗、或作为疫苗的辅剂，其中互相成分是如细胞因子，特别是细胞因子（IL－3、IL－6）或细胞因子分泌诱导剂如类脂衍生物，例如描述于EP 0309411，特别是在实施例1中的化合物，也称为MRL 953；

特殊的免疫抑制，如其中互相成分为亲免疫结合（immunophilinbinding）免疫抑制剂如环孢菌素（如环孢菌素A）、子囊霉素（如FK506）、或雷怕霉素（如WO 94/09010描述的雷怕霉素或其衍生物如40－O－羟乙基－雷怕霉素）；

通过抗炎细胞因子的缓释治疗或预防自身免疫性疾病和炎性疾病，如IL－6、IL－10或TGFβ，或干扰素如IFN－β 1或Betaseron，或可溶性细胞因子受体或细胞因子受体拮抗剂如细胞因子IL－I、TNFα或IL－4；

通过IgE的高亲和力受体（FcERI）的可溶性α链的缓释治疗或预防过敏性疾病；

癌症治疗，如用 otreotide 、细胞因子特别是白细胞介素；

选择靶向，如用于治疗利什曼病、真菌感染、酶沉积病（泰－萨二氏病、高歇氏病）；

AIDS 或 ARC 治疗；

接种如用破伤风类毒素疫苗；

造血，例如其中的活性成分是红细胞生成素；

对炎症关节进行关节间注射，其中活性成分为抗炎药，优选口服不能生物利用或有非常短的半衰期如 IL － 1 β 转化成酶抑制剂、含金属蛋白酶抑制剂.

一种提高哺乳动物对疫苗的免疫反应的方法，包括给需要接种的哺乳动物使用有效剂量的 GM － CSF 和疫苗，这描述于国际 PCT 申请 WO 94/01133 . 但是，不能按照本发明的方式较好地阻止 GM － CSF 释放，在较长的时期内活性化合物的释放接近常数，通过这种方法重复使用 GM － CSF 的次数可以降低.

本发明特别提供了一种存在于聚合物中的药物活性化合物的药物组合物，它表现为非水解表面溶蚀，用于白细胞介素或 CSF 的非肠道给药，特别是存在于本文定义的聚合物中.

本发明也提供可一种给患者使用此类组合物的方法，包括为需要此治疗的患者非肠道给药.

本发明的贮库制剂可用于引人的特殊药物化合物，治疗其已知症状.

所用药物化合物及贮库制剂的准确量依赖于若干因素如所治疗的疾病、所需的治疗时间、药物化合物的释放速率及聚（碳酸亚乙酯）的降解性.

所需制剂可用已知方式制备．所需药物活性制剂的量及其释放速率可根据已知的技术在体外或体内测定，如特别活性制剂的血浆浓度保持在可接受水平的时间．基质的降解性也可通过体外或优选体内的技术测定，例如在特定的时间后测定皮下组织中基质物质的量．

本发明的贮库制剂可用如微粒的形式通过口、鼻或肺给药，优选皮下、肌注或静脉给药，特别是在适当液体载体中以混悬液或植入片的形式（如皮下）．

如果聚合物基质在 1 、 2 或 3 星期或 1 个月后充分降解，则本发明贮库制剂的重复给药会起作用．

本发明的聚（碳酸亚乙酯）基质的优点是在药物化合物释放期间聚合物链降解为小分子部分，由体液将它从用药位置转运走．

含有优选的化合物 octreotide 药物的实例为治疗肢端肥大症，在含有微粒的非肠道液体贮库制剂中肽占（共）聚合物基质重量的至少 0.1（优选 0.5 ）至 20 %，优选 2.0 至 10，特别优选 3 至 6 %．治疗 1 个月所需 octreotide 的总量在肢端肥大症中为 20 至 30mg 、在乳腺癌中达到 100 至 200mg ．

肽从微粒中释放的时间可以是 5 天至约 2 个星期或更长．

一般缓释制剂包含（共）聚合物载体中的 octreotide ，当给兔或鼠使用剂量为 2mg/kg 动物体重的 octreotide ，在较长的时间内 octreotide 的血浆浓度至少为 0.3ng/ml 并优选小于 20ng/ml ．

本发明药物组合物可含有其它添加剂，它也优选置于（共）聚合物中如自由基清除剂，特别是过氧自由基阴离子 O_2^- 的清除剂．这种清除剂（甲萘醌或维生素 C ）的存在降低了聚（碳酸亚乙酯）的降解速率（图 7 ）．

添加剂的另一种类型是羟基自由基的清除剂，羟基自由基可能在过

氧自由基阴离子 O_2^- 的影响下产生，例如多元醇，特别是糖醇，尤其是甘露醇．此添加剂对试验动物的体重也有有利的影响，此动物所用的药是微胶囊 IL - 3．如果没有这种添加剂，会延缓体重的增加．当组合物是微粒形式时同一添加剂或其它添加剂可加人到微粒的外部，因为它对微粒悬液的稳定性有有利的影响，可防止絮状物和沉淀物的产生．

如果存在添加剂，其含量优选占制剂总重量的 1 至 90 %．

过氧自由基阴离子 O_2^- 的影响有利于体外和体内的物质降解，如图 8 所示．残余物质的降解曲线接近直线并有不同的斜率，这是因为体内及体外的降解条件不同．每单位时间降解物质的量几乎是常数．

药物活性物质（如人 IL - 3）在过氧自由基阴离子 O_2^- 的影响下，它在体内释放的曲线与降解曲线一样接近直线（图 10），这意味着单位时间内释放的药物化合物的量几乎是常数．

图 11 记录了体内人 IL - 3 的释放和体内物质降解，表明体内物质降解和药物释放的相互关系为 1： 1．

实施例 1 - 5：使用由二乙基锌和水制备的催化剂合成聚（碳酸亚乙酯）的一般方法

特定试验中反应物、溶剂、催化剂等的量见表 1．

将 200ml 干燥二㗁烷及 19.5g （ 158mmol ）二乙基锌置于氮气氛下的 750ml 烧瓶中．烧瓶装有机械搅拌器、滴液漏斗、温度计和一个氮气人口．滴液漏斗上装有二氯化钙管．在冰浴中将溶液冷却至 10 ℃并慢慢加人存在于二㗁烷中的水 2.7ml （见表 1），保持温度在 10 - 15 ℃．在室温将反应混合物再搅拌 45 分钟，直到无色溶液变为浅黄色．将催化剂溶液转移至高压釜中，用 40g 二氧化碳处理并在 125 ℃加热表 1 中指明的时间．然后将混合物冷却至室温并加人 560g(12.7mol) 二氧化碳，

随后在 1 小时内慢慢加入 132g(3mol)环氧乙烷.反应进行的时间见表 1．此后，在几小时内慢慢降低压力．用二 恶烷稀释粘浆状产品，将二α恶烷溶液到进 0.25M 的氯化氢水溶液中形成沉淀．将沉淀溶于适量的二氯甲烷（ 2 - 4 升）中，用 0.5M HCl 水溶液（ 2x ）和水（ 1x ）洗涤．用无水硫酸钠干燥溶液并根据溶液的粘度将溶液蒸发至 0.5 至 1.5 升．将二氯甲烷溶液到入 4 倍体积的甲醇中沉淀产品．过滤白色产品并在 0.5 毫巴/50 ℃干燥过夜．用丙酮再沉淀粗产品使其进一步纯化．由于碳酸亚乙酯的含量不同，故除了在 3.65 、 3.73 、 4.29 及 4.37ppm 的信号相对强度外所有的产品有相同的 1H-NMR 谱．

表 1： 制备聚（碳酸亚乙酯）类试验

实施例	环氧乙烷 [mol]	[mol]	CO_2 [mmol]	$Zn(C_2H_5)_2$二α恶烷 [ml]	温度 [℃]	时间 [h]
1	3	13.6	158	300	50	64
2	3	9.1	158	500	20	64
3	3	13.6	158	300	20	240
4	3	13.6	158	300	20	40
5	3	13.6	158	300	20	22
6	3	13.6	238	300	50	64

所有试验在 1.0 升高压釜 NB2 中进行．所有试验中水：二乙基锌摩尔比为 0.95．催化剂用 40g 二氧化碳在 125 ℃预处理 1 小时，实施例 1 除外（ 10 小时）．

表 2：合成聚（碳酸亚乙酯）类的部分物理性质

实施例	Mw [kDa]	Mn [kDa]	Mw/Mn	Tg [°C]	η_{inh} [dl/g] 碳酸亚乙酯在 $CHCl_3$ 中 a) 含量(%)
1	141.9	32.2	4.40	19.3	0.6087
2	627.3	133.5	4.70	23.5	1.4691
3	477.0	83.6	5.71	18.7	1.2791
4	758.0	97.5	7.77	20.6	1.7590
5	721.6	80.7	8.95	22.9	2.44 b)90
6	310.9	103.1	3.02	20.1	88

a) 如果不特别指明，则在 20 ℃浓度为 10mg/ml

b) 浓度为 1mg/ml

实施例 7 - 11：使用由二乙基锌和二醇制备的催化剂合成聚（碳酸亚乙酯）类的一般方法

1. 催化剂的制备

将 200ml 干燥二 n恶烷置于氮气氛下 750ml 干燥的四颈烧瓶中．通过玻璃注射管加人 19.50g （ 158mmol ）二乙基锌．烧瓶装有机械搅拌器、滴液漏斗、温度计及氩气人口．滴液漏斗中装有 100ml 干燥二 n恶烷并装有氯化钙管．然后将仪器置于氩气气流中．在氩气流中， 9.00(145 mmol, 0.92 克分子当量) 的新蒸馏的、干燥的甘醇加人（在分子筛上）到滴液漏斗中的二 n恶烷中．机械搅拌烧瓶，在氩气氛下用冰浴将温度降低至 10 ℃．在 30 分钟内将甘醇的二 n恶烷溶液滴加至搅拌的二乙基锌的二

噁烷溶液中，这期间保持温度在 10 - 14 ℃. 加入 1， 2 -亚乙基二醇溶液的同时观察到乙烷气体的放出和沉淀的产生. 加毕移去冰浴并将混合物再搅拌 60 分钟，同时使其升至室温. 然后在氩气氛将多相混合物转移至高压釜（ 1 升高压釜 NB2 ）中. 在搅拌的同时，高压釜充入约 40g （ 0.9 mol ）二氧化碳并在 125 ℃加热 1 小时，用二氧化碳预处理催化剂.

2. 聚合反应

将装有预处理的催化剂的高压釜冷却至室温并再充入 560g （ 12.7 mol ）二氧化碳. 然后在 1 小时内通过慢慢注射将 132g （ 3 mol ）环氧乙烷（ 99.8 % ）加入到高压釜中的搅拌的混合物中. 加毕，将高压釜加热至表 3 中指明的温度并在此温度在指定的时间内搅拌混合物.

3. 后处理

将高压釜冷却至室温并慢慢将压力降至常压. 用 7 升二氯甲烷吸收白色粘浆状产品，加入 1035ml 0.4M HCl 溶液，在室温将混合物搅拌 3 小时. 将两相分离并将有机层用 3 升 0.5M HCl 洗涤两次，用 4.5 升水洗涤两次. 再用 120 g 硫酸钠干燥二氯甲烷溶液并浓缩至最后体积为约 2 升. 将此溶液慢慢加入 6 升甲醇中沉淀产品. 在 40 ℃真空干燥沉淀 16 小时得到粗品聚合物，在按以下方法进一步纯化:

将粗品溶于二氯甲烷中，在 15 分钟内将溶液到入 5 倍体积的丙酮中沉淀产品. 在 40 ℃真空干燥沉淀 16 小时得到相应的聚（碳酸亚乙酯）. 产品的物理性质列于表 4 . 所有产品在 1750 和 1225cm-1 有强 IR 吸收. 碳酸亚乙酯单元的 1H - NMR 信号在 4.37 ppm .

表 3： 使用由二乙基锌和二醇合成聚（碳酸亚乙酯）类

实施例	环氧乙烷 Oxide [mol]	CO_2[mol]	溶剂 a) [ml]	$(C_2H_5)_2Zn$ [mmol]	二醇 b) [mmol]	反应温度 [°C]	反应时间 [hrs]
7	3.0	13.6	300	158	145	20	96
8	3.0	13.6	300	158	145	50	96
9	3.0	13.6	300	158	145	60	96
10	3.0	13.6	300 c)	158	145	50	144
11	3.0	13.6	300	158	145 d)	50	96

a) 二噁烷（如不特别指明）

b) 甘醇（如不特别指明）

c) 用四氢呋喃代替二噁烷作为溶剂

d) 用 1，4－丁二醇代替甘醇

表 4：用由二乙基锌和二醇制备的催化剂合成的聚（碳酸亚乙酯）类的部分物理性质

实施例	Mw [kDa]	Mn [kDa]	Mw/Mn	Tg [°C]	η_{inh} [dl/g] 在 $CHCl_3$ 中 a)	碳酸亚乙酯含量(%)
7	-	-	-	16.7	2.88 b)	98
8	328.0	149.0	2.20	16.4	0.97	95
9	207.0	103.0	2.00	21.2	0.65	92
10	231.0	83.8	2.76	32.6	0.72	96
11	110.0	53.4	2.06	31.1	0.49	90

a) 如不特别指明，则在 20 ℃且浓度为 10mg/ml

b) 浓度为 1 mg/ml

实施例 12 ： 使用由二乙基锌和间苯三酚制备的催化剂合成聚（碳酸亚乙酯）的试验方法

1. 催化剂的制备

将 200ml 干燥二 α恶烷置于氮气氛下 750ml 干燥的四颈烧瓶中. 通过玻璃注射管加入 19.60g （ 158.7mmol ）二乙基锌. 烧瓶装有机械搅拌器、滴液漏斗、温度计及氩气入口. 滴液漏斗中装有 100ml 干燥二 α恶烷并装有氯化钙管. 然后将仪器置于氩气气流中. 在氩气流中， 13.34 (105.8 mmol, 0.92 克分子当量) 干燥的间苯三酚加入（在分子筛上）到滴液漏斗中的二 α恶烷中. 机械搅拌烧瓶，在氩气氛下用冰浴将温度降低至 10 ℃. 在 30 分钟内将间苯三酚的二 α恶烷溶液滴加至搅拌的二乙基锌的二 α恶烷溶液中，这期间保持温度在 10 - 14 ℃. 加入间苯三酚溶液的同时观察到乙烷气体的放出和沉淀的产生. 加毕移去冰浴并将混合物再搅拌 30 分钟，同时反应升至室温. 然后在氩气氛将多相混合物转移至高压釜（ 1 升高压釜 NB2 ）中. 在搅拌的同时，高压釜充入约 40g （ 0.9 mol ）二氧化碳并在 125 ℃加热 1 小时，用二氧化碳预处理催化剂.

2. 聚合反应

将装有预处理的催化剂的高压釜冷却至室温并再充入 560g （ 12.7 mol ）二氧化碳. 然后在 1 小时内通过慢慢注射将 132g （ 3 mol ）环氧乙烷（ 99.8 % ）加入到高压釜中的搅拌的混合物. 加毕，高压釜在 21 ℃搅拌 260 小时.

3. 后处理

将高压釜冷却至室温并慢慢将压力降至常压. 用共 4 升二氯甲烷吸收白色粘浆状产品，加入 1035ml 0.4M HCl 溶液，在室温将混合物搅拌 3 小时. 将两相分离并将有机层用 1.5 升 0.5M HCl 洗涤两次，用 2 升水洗涤两次. 再用 120 g 硫酸钠干燥二氯甲烷溶液并浓缩至最后体积为约 1 升. 将此溶液慢慢加入 3 升甲醇中沉淀产品. 在 40 ℃真空干燥沉淀 16 小时得到粗品聚合物，在按以下方法进一步纯化:

将粗品溶于二氯甲烷中，在 15 分钟内将溶液到入 5 倍体积的丙酮中沉淀产品. 再将沉淀溶于二氯甲烷中，从甲醇中再沉淀并在 40 ℃真空干燥沉淀 16 小时得到相应的聚（碳酸亚乙酯）.

产品的物理性质:

Mw=258000 Da, Mn=35600 Da, Tg=15.4 ℃.

IR: 在 1751 和 1225cm-1 强吸收.

根据 1H - NMR，产品的碳酸亚乙酯含量为 96 %.

实施例 13: 使用由二乙基锌和丙酮制备的催化剂合成聚（碳酸亚乙酯）的试验方法

132g(3 mol)环氧乙烷与 600g （ 13.6mol ）二氧化碳在 50 ℃使用由 8.43g （ 145.16 mmol ）丙酮和 19.62g （ 159mmol ）二乙基锌制备的催化剂共聚反应 96 小时.

除了用丙酮代替二醇制备催化剂外，催化剂的制备和聚合反应按照与实施例 7 - 11 相似的方法进行.

这样制得的聚（碳酸亚乙酯）的碳酸亚乙酯含量为 93 %并具有如下性质:

Mw=233kDa, Mn=109kDa, Mw/Mn=2.14, Tg=22.4 ℃.

实施例 14：合成末端基团硬脂酰化的聚（碳酸亚乙酯）

将 1 g 聚（碳酸亚乙酯）（Mw=153000 Da, Mn=68900 Da, Tg=29.1 ℃.）溶于 30 ml 干燥的二氯甲烷中. 随后用 0.98g（12.38 mmol）吡啶和 10 g（33.0 mmol）硬脂酰氯处理溶液. 在室温将反应混合物搅拌 48 小时，然后用 50 ml 二氯甲烷稀释并用 2x150ml 饱和碳酸氢钠和水连续洗涤. 用无水硫酸钠干燥有机相，通过将二氯甲烷溶液滴加进 300 ml 正己烷中沉淀产品. 这样得到的粗品通过溶于二氯甲烷并从 3 倍体积的乙醚中沉淀进一步纯化. 最后，在 40 ℃真空干燥产品 16 小时得到末端基团硬脂酰化的聚（碳酸亚乙酯）.

Mw=144000 Da, Mn=71000 Da, Tg=25.6 ℃.

实施例 15：末端基团乙酰化的聚（碳酸亚乙酯）的合成

将 1 g 聚（碳酸亚乙酯）（Mw=153000 Da, Mn=68900 Da, Tg=29.1 ℃.）溶于 10 ml 干燥的二氯甲烷中. 加入 0.98g（12.38 mmol）吡啶，随后加入 10.08 g（98.7 mmol）乙酸酐. 在室温将反应混合物搅拌 120 小时，然后用 50 ml 二氯甲烷稀释并缓慢加入到 200 ml 饱和碳酸氢钠中. 混合物搅拌 30 分钟然后分离两相溶液. 再用 150ml 饱和碳酸氢钠和最后用水洗涤有机相. 用无水硫酸钠干燥二氯甲烷溶液，通过将此溶液滴加进 300 ml 乙醚中沉淀产品. 沉淀再溶于二氯甲烷并从乙醚中再沉淀. 在 40 ℃真空干燥产品 16 小时得到具有末端乙酸酯基团的聚（碳酸亚乙酯）.

Mw=150000 Da, Mn=69100 Da, Tg=26.8 ℃.

实施例 16：通过用沸水处理纯化聚（碳酸亚乙酯）

将 1 g 聚（碳酸亚乙酯）（实施例 8，Mw=328000 Da, Mn=149000 Da, Tg=16.4 ℃.）切割成小片并在沸水中搅拌 2 小时. 除去水并换成新鲜

水，再将其加热至沸点． 3 小时后，分离聚合物片并在 40 ℃真空干燥 16 小时．得到的产品具有以下物理性质： Mw=340000 Da, Mn=148000 Da, Tg=28.3 ℃．这样，在不改变聚合物分子量的前体下，观察到玻璃化温度急剧增加．

实施例 17： 含 1 % hIL-3 药物填料的组合物（微粒）

1. 含微粒药物的制备

将 1g 聚（碳酸亚乙酯）（实施例 8 （ PEC ） Mw=328000 ）边搅拌边溶于 10ml 二氯甲烷，随后加入溶于 0.6ml 水中的 12.1mg 人白细胞介素 3 （ hIL-3 ）．用 Ultra-Tarrax 在 20000 rpm 彻底搅拌混合物 1 分钟（ = 内 W/O －相）．在 50 ℃将 1 g 明胶 A 溶于 2000ml 去离子水中并将溶液冷却至 20 ℃（外 W 相）．彻底搅拌 W/O －相及 W －相．这样内 W/O －相均匀分散在外－ W －相中形成细的小滴．所得三相乳液缓慢搅拌 1 小时．以此将二氯甲烷蒸发，由内相的小滴形成微粒并硬化．

微粒沉积后，吸除上清液并通过真空过滤或离心回收微粒，用水清洗除去明胶．最后，微粒通过以甘露醇作填充剂冷冻干燥或在真空烘箱中干燥 72 小时（无甘露醇的制剂），过筛（筛目大小为 0.125mm ）得到最佳产品．

2. 空白对照剂

将 1g 实施例 8 的 PEC， Mw = 328000 边搅拌边溶于 10ml 二氯甲烷（内 O －相）．在 50 ℃将 1g 明胶 A 溶于 2000ml 去离子水中并将溶液冷却至 20 ℃（ =外 W 相）．彻底搅拌 O －相及 W －相．这样 O －相均匀分散在外 W －相中形成细的小滴．所得乳液缓慢搅拌 1 小时并按上述方法处理．

实施例 18 － 26：

此后描述的所有明胶制剂是使用按照实施例 8 表 3 合成的 PEC 类制备并以类似于实施例 16 的方法纯化。它们的 Mw 为 300000 至 450000，碳酸亚乙酯的含量大于 94 %， Tg 为 18 至 50 ℃。

实施例 18： 含 0.2 % hIL-2 填料的组合物（微粒）

将 2.9mg 人白细胞介素 2 （ hIL-2 ）溶于 1.5ml 水中，并按照实施例 17 制备含 IL - 2 的微粒。微粒通过以甘露醇作填充剂冷冻干燥并过筛（筛目大小为 0.125mm ）得到最终产品。

实施例 19： 含 0.2% hIL-2 填料（无水）的组合物（微粒）

按照实施例 18 制备制剂，但不同的是将 2.9mg 人白细胞介素 2 直接分散在有机相中（ PEC 溶解于二氯甲烷）。

实施例 20： 含 0.8% hIL-3 填料的组合物（植人剂）

1. 压模

将含有 100 % （ w/w)聚(碳酸亚乙酯 X 空白对照剂)、 99 % （ w/w ）聚（碳酸亚乙酯）和 1 % （ w/w ）人白细胞介素 3 或 79.2%（ w/w ）聚（碳酸亚乙酯）、 20 % （ w/w ）甘露醇及 0.8%（ w/w ）人白细胞介素 3 的微粒 25mg 在 60 - 70 ℃、 160 巴压模 3 分钟，制成直径 5 mm 的植人剂（片剂）。在用于体外或体内药物释放试验前，此片剂在 4 ℃装于密闭的玻璃瓶中。

2. 体外药物释放试验

无甘露醇白细胞介素 3 、含甘露醇白细胞介素 3 及空白对照剂的三种片剂 37 ℃在合成培养基中摇动。该培养基含有 2.5%（ v/v ） N - [2 - 羟乙基] - 哌嗪 - N′ - [2 - 乙基磺酸]（ 1m ）、 10 %（ v/v ）小牛血清和 2 %（ v/v)青霉素/链霉素溶液.在第 0.5 、 1 、 2 、 5 小时及 1 、 2 、 3 、 7 、 14 、 20 小时从培养基中取样，随后更新培养基。样品中

人白细胞介素 3 的含量通过 ELISA 测定.

3. 体内药物释放试验

将最佳状态的雄性鼠通过吸入麻醉剂麻醉，将人白细胞介素 3 制剂和空白对照剂的片剂植入每个鼠的皮下囊中.在第 1 、 4 、 7 、 14 、 21 天后，通过吸入过量的麻醉剂将鼠处死. 取出残留的片剂，除去附着的组织并干燥.通过差重法测定片剂的物质损失.随后，通过 HPLC 和 ELISA 测定残留片中人白细胞介素 3 的含量.

实施例 21： 含有 0.0002%-2% hIL-2 填料的组合物（ w/o/w 微粒）

将 4 gPEC 边电磁搅拌边溶于 80 ml 二氯甲烷中. 向此溶液中加入溶于 6 ml 蒸馏水或含几滴乙醇的水中的适量的 IL - 2 （ 113.2 mg 对应 2 %、 11.32 mg 对应 0.2 % 等）中. 用 Ultra-Turax 彻底混合混合物，将 IL - 2 溶液分散于聚合物相（ = 内 W/O 相）中. 在 50 ℃将 1 g 明胶 A 溶于 200ml 1/15M 磷酸盐缓冲液（ pH=7.4 ）中并将溶液冷却至 20 ℃（ = 外 W 相）. 彻底混合 W/O - 相及 W - 相. 这样内 W/O - 相均匀分散在外 - W - 相中形成细的小滴. 所得三相乳液缓慢搅拌 1 小时. 以此将二氯甲烷蒸发，由内相的小滴形成微粒并硬化.

微粒沉积（或离心）后，吸除上清液并通过真空过滤回收微粒，用水清洗除去明胶. 最后，微粒在真空烘箱中干燥 24 小时，过筛得到最终产品.

用 HPLC 和生物试验测定的包囊效率为 10 至 100 %.

实施例 22： 含 0.0002%-2% IL-2 填料的组合物（ s/o/w 微粒）

按照实施例 21 制备制剂，不同的是 IL - 2 不是溶于水中. IL - 2 不用溶解，药物直接分散到聚合物相（ = O - 相）中. 用 HPLC 和生物试验测定的包囊效率为 10 至 100 %.

注意：聚合物、二氯甲烷、水及药物的量可在大范围内变化而不改变产品的性质．可制得较高的载药量为 20 %．在外相中，用聚乙烯醇等其它乳化剂代替明胶，乳化剂/缓冲剂的浓度也可变化．分离和干燥方法可用其它熟知的制药技术代替，如过滤、冻干或喷雾干燥．

实施例 23：含 1 % hGM-CSF 填料的组合物（ w/o/w 和 s/o/w 微粒）

按照实施例 21 和 22 的方法制备 s/o/w 和 w/o/w 制剂．但 w/o/w 制剂的包囊效率为 60 %，而 S/O/W 制剂具较低的包囊效率．

实施例 24：含 1 至 10 % Octreotide-双羟萘酸盐（ SMS - PA ）填料的组合物（ w/o/w 和 s/o/w 微粒）

按照实施例 19 和 20 的方法制备．但 SMS - PA 不溶于水．因此，对于 W/O/W 制剂药物是分散在而不是溶解在水中．通过 HPLC 测定的包囊效率为 20 至 100 %．

实施例 25：含 1 至 10 % Octreotide-乙酸盐填料的组合物（ w/o/w 和 s/o/w 微粒）

按照实施例 21 和 22 的方法制备．通过 HPLC 测定的包囊效率为 2 至 40 %，这明显比亲脂性的 SMS - PA 低．在使用冻干的活性化合物物质后，在 S/O/W 制剂中得到较高值（较小的药物颗粒）．

实施例 26：从兔体内微粒及兔和鼠体内植入剂中释放 Octreotide 双羟萘酸盐（ SMS - PA ）

给雄性兔（灰鼠杂种，体重约 3 kg ）皮下植入聚（碳酸亚乙酯）片或注射聚（碳酸亚乙酯）微粒（含药量 1.95%），用量约为 2 mg 药物每公斤体重．给雄性鼠（ Wistar，体重约 375 g ）皮下植入片．每个鼠和兔的量分别是 40 和 300 mg 含药的聚合物，以微粒的形式，将其分别压成植入剂或作为混悬剂使用．

鼠和兔的植入片的直径分别为 0.5 和 1 cm，并按照实施例 20 制备.

为测定药物释放，在第 14 和 21 天分别收集鼠和兔的血样，并通过放射免疫测定法及 HPLC 测定植入剂中的药物残余物.

可以发现正如高分子量物质 hIL - 3（图 11），聚（碳酸亚乙酯）的物质损失和 SMS - PA 的释放为直线关系（图 13）. 给兔用药后 3 星期植入物质有最大的降解值 75 %，给鼠用药后 2 星期植入物质有最大的降解值 95 %. 炎性反应（包括多形核白细胞及其它细胞的侵入）是聚（碳酸亚乙酯）发生生物降解的前提. 可以预计炎性反应的过程是种特异的，引起药物血浆水平的种特异性现象. 此发现针对 SMS - PA（图 12）. 聚（碳酸亚乙酯）在鼠体内比在兔体内生物降解更快. 兔的 SMS - PA 的血浆水平缓慢地增加，在约地 9 天达到恒定的释放状态，并持续 21 天.

实施例 27： 含 0.0002%-2% rhIL - 6 填料的组合物（w/o/w 微粒）

将 4 gPEC 边电磁搅拌边溶于 80 ml 二氯甲烷中. 向此溶液中加入溶于 6 ml 蒸馏水或含几滴乙醇的水中的适量的 rhIL - 6（113.2 mg 对应 2 %， 11.32 mg 对应 0.2 % 等）. 用 Ultra-Turax 彻底混合混合物，将 IL - 6 溶液分散于聚合物相（＝内 W/O 相）中. 在 50 ℃将 1 g 明胶 A 溶于 200ml 1/15M 磷酸盐缓冲液（ph=7.4）中并将溶液冷却至 20 ℃（＝外 W 相）. 彻底混合 W/O - 相及 W - 相. 这样内 W/O - 相均匀分散在外 - W - 相中形成细的小滴. 所得三相乳液缓慢搅拌 1 小时. 以此将二氯甲烷蒸发，由内相的小滴形成微粒并硬化.

微粒沉积（或离心）后，吸除上清液并通过真空过滤回收微粒，用水清洗除去明胶. 最后，微粒在真空烘箱中干燥 24 小时，过筛得到最终产品.

用 HPLC 和生物试验测定的包囊效率为 10 至 100 %.

实施例 28： 含 0.0002%-2% rhIL - 6 填料的组合物（ s/o/w 微粒）

按照实施例 27 制备制剂，不同的是 IL - 6 不是溶于水中． IL - 6 不用溶解，药物直接分散到聚合物相（ = O - 相）中．用 HPLC 和生物试验测定的包囊效率为 10 至 100 %．

注意： 聚合物、二氯甲烷、水及药物的量可在大范围内变化而不改变产品的性质．可制得较高的载药量为 20 %．在外相中，用聚乙烯醇等其它乳化剂代替明胶，乳化剂/缓冲剂的浓度也可变化．分离和干燥方法可用其它熟知的制药技术代替，如过滤、冻干或喷雾干燥．

实施例 29 - 31：使用 IL - 6 治疗 TNF α和/或 IL - 1 介导的疾病

实施例 29：多发性硬化的动物模型： Lewis 鼠试验诱发的过敏性脑脊髓炎模型的慢性复发（ CR - EAE ）

本领域试验诱发的过敏性脑脊髓炎（ EAE ）是人多发性硬化的很好的研究试验模型．[Paterson, ADV. IMMONOL. 5(1966) 131-208; Levine et al., AM. J. PATH. 47(1965) 61; Mcfarlin et. al., J. IMMUNOL. 113(1974) 712; Borel, TRANSPLANT & CLIN. IMMUNOL. 13(1981) 3]．给鼠注射其它种的神经组织和佐剂，用所得过敏反应导致的鼠神经损伤模拟多发性硬化产生的自身免疫损伤．鼠变得部分或完全麻痹，测定用药或不用药时疾病的严重程度．一些药物如甾类和免疫抑制剂有减慢疾病发作的活性，但一旦染上疾病它们不能预防其复发．

因此，试验诱发的过敏性脑脊髓炎模型的慢性复发（ CR - EAE ）[Feurer, et. al., J. NEUROIMMUNOL: 10(1985) 159-166]被看作特别符合需要的模型，它能非常接近地模拟在治疗多发性硬化患者中的实际困难．在此模型中，疾病是通过注射豚鼠脊髓和富含结核分支杆菌的福氏佐剂诱发的．一般 75 - 80 %的被致敏鼠感染 CR - EAE，在开始的 40

天里复发 2 - 3 次. 60 - 80 天后，约 50 % 患 CR - EAE 鼠进一步复发，所有病例中完全恢复率只有 35 %. 其余的 65 % 疾病进一步发展. 在从第一次疾病发作恢复后，第 16 天开始药物治疗.

重组人白细胞介素 6 （ rhIL-6, Sandoz ）溶于生理盐水中，在第 16 天开始用 10 微克 IL - 6 （约 50 μ g/kg ）隔天每只鼠腹膜内注射. 对照组和 IL - 6 用药组在第 11 - 14 天有普遍的严重疾病发作（急性）. 严重程度的评分为 0 = 无疾病至 4 = 动物完全麻痹，对照组平均为 3.0 而 IL - 6 组平均 3.2 . 从第 16 天至第 30 天（共 7 次给药）隔天使用 IL - 6 使疾病几乎完全抑制. 在第 16 天后， 5 只对照鼠全部第二次发作，其平均严重发病率为 1.8 ，在第 22 - 29 天第三次发作. 在 IL - 6 用药组中未观察到其它发作.

实施例 30： 关节炎动物模型: 对患严重综合免疫缺乏（ SCID ）鼠由疏螺旋体属诱发的关节炎

莱姆关节炎（或莱姆病关节炎）代表了一类独特的慢性关节炎，因为诱发因素已知是必然的. 此病的主要特征是由蜱生螺旋体疏螺旋体属 burgdorferi 感染诱发. 莱姆病关节炎病人滑膜损伤特征与类风湿关节炎患者的滑膜非常接近. 在两组病人中，观察到滑膜衬里细胞 hyperthrophy 、滑膜细胞增生、血管增殖及在滑膜衬里区域单核细胞侵润. 发现很多浆细胞、高内皮小静脉、散布巨噬细胞及很少的树突细胞有强 MHC II 类抗原表达. 此外，在多种关节炎疹患者的滑膜液中发现了 IL - 1 、 IL - 6 和 TNF α等细胞因子，这表明这些细胞因子可能与关节损伤的病因有关. 最近，用缺乏功能性 T 和 B 细胞的 SCID 鼠建立了莱姆病关节炎的鼠模型（ M. M. Simon, et. al. (1990) Immunology Todday 12: 11 ）. 用疏螺旋体属 burgdorferi 感染免疫缺乏的鼠导致明显的且

持续的低关节炎.疏螺旋体属诱发 SCID 鼠关节炎,对皮质甾类（ 30mg/kg 皮下使用强的松）有反应，而对剂量达 30mg/kg s.c.如 SIM （环孢菌素 A ）等免疫抑制剂无反应．这是细胞因子造成的（也包括诱发因素已知确定的其它类型）关节炎的较好的模型.

通过尾底部注射给 6 星期大的 C.B - 17 SCID 鼠（ SCID 突变的纯合子，得自 Bomholtgard, Denmark, 5-6 只动物/组）接种 100 mio.疏螺旋体属 burgdorferi 有机体．有免疫能力的 C.B - 17 鼠（相同来源）作为对照动物．注射疏螺旋体属 burgdorferi 后它们不得任何疾病．用生理盐水稀释重组人 IL - 6 （ rhIL-6, Sandoz, 保存液浓度为 5mg/ml ），并每星期 5 次给药，共腹膜内注射 17 次，剂量为 10 微克每只鼠．以双盲形式每天观察小鼠胫跗及尺腕关节的关节炎临床病症．按照下列参数临床评分:

- \- 无病症
- ? 有疑问
- (+) 关节变红
- \+ 轻度肿胀
- + + 中度肿胀
- + + + 胫跗和尺腕关节严重肿胀.

在临床关节炎的高峰值，杀死小鼠，用 Schaffer 氏溶液固定关节，置于 9100 塑料中并用苏木紫伊红染色.

组	第 n 天的临床病症（肿胀的关节数/关节总数）					
	13	14	15	16	17	20
对照	0/30	0/30	0/30	0/30	0/30	0/30

SCID. IL-6	6.5/36	12.5/36	15/36	21/36	30/36	35/36
% 患关节炎	18 %	35 %	42 %	58 %	83 %	97 %
SCID. IL-6 治疗	4/30	3.5/30	11/30	7.5/30	12/30	10.5/30
% 患关节炎	13 %	12 %	37 %	25 %	40 %	35 %

未用 IK - 6 治疗的 SCID 小鼠因疏螺旋体属 burdorferi 感染在抗原注射约第 13 天后产生严重的关节炎. 在所有病鼠中, 低剂量的 rhIL-6 以平均 60 - 75 %降低关节炎的严重程度.

实施例 31: 败血症休克的鼠模型

因为其广泛地用作人败血症休克的模型, 故决定在小鼠内毒素休克模型中使用 d - 半乳糖胺致敏小鼠研究 IL - 6 的效果. 我们的方法和结果如下:

雌性 OF1 小鼠重 18 - 22g, 腹膜内注射含 0.15 mg/kg 脂多糖内毒素 (LPS) 和 500 mg/kg d - 半乳糖胺的 PBS 溶液 0.2 ml. 将每 10 只小鼠分为一组, 并作如下处理:

实验 1

时间	11:00	14:00	16:00
组 1	PBS	LPS + d-GAL	PBS
组 2	IL-6 (50 μg)	LPS + d-GAL	PBS
组 3	PBS	LPS + d-GAL + IL-6 (50 μg)	PBS
组 4	PBS	LPS + d-GAL	IL-5 (50 μg)

实验 2

时间	11:00	14:00	16:00
组 1	PBS	LPS + d-GAL	PBS
组 2	IL-6 (50 μg)	LPS + d-GAL	PBS
组 3	PBS	LPS + d-GAL + IL-6 (100 μg)	PBS

组 4	PBS	LPS + d-GAL + IL-6 (20 μg)	PBS
组 5	PBS	LPS + d-GAL + IL-6 (5 μg)	PBS
组 6	PBS	LPS + d-GAL + IL-6 (0.8 μg)	PBS
组 7	PBS	LPS + d-GAL + IL-2 (100 μg)	PBS
组 8	PBS	LPS + d-GAL + IL-4 (50 μg)	PBS
组 9	PBS	LPS + d-GAL	IL-6 (50 μg)

rhIL-6 （ ILS 969, Sandoz ）、 rhIl-2 （ Sandoz ）及 rhIL-4 （ Sandoz ）用 PBS 稀释. 所有注射（体积 0.2 ml ）为腹膜内注射. 在组 3 （试验 1 ）和组 3 至 8 （试验 2 ） IL - 6 和 IL - 2 稀释入 LPS/d-GAL 溶液中，以便小鼠得到单剂量 0.2 ml 注射. 括号中的数字表示每只小鼠白细胞介素的用药量. 需要多剂量 PBS 控制由于应激反应产生的组间变化性，此应激反应因在 LPS 使用前或后的不同时间的操作产生.

观察小鼠存活 48 小时. 我们使用卡方检验进行统计计算. 如图 1 所示，在使用 LPS 24 小时后 10 只对照小鼠中有 9 只死亡. 在 LPS 注射前 3 小时或后 2 小时用 IL - 6 治疗分别将死亡率降低至 60 % （ p=0.12 ）和 70 % （ p=0.26 ）. 另一方面，在使用 LPS 同时使用 IL - 6 将死亡率降低至 10 % （ p=0.01 ）. 保护效果持续很长时间，因为 48 小时后组 3 中的死亡率只轻微增加（即增至 30 % ），与对照组相比仍有显著的保护（ p<0.01 ）. 组 4 的死亡率在 70 % 至 80 %，而在组 1 和 2 中未观察到

变化.

基于这些结果，我们试验不同剂量 IL - 6 的效果. 在注射 LPS 的同时使用 IL - 6 ，因为按照第一个试验这是最佳的时间. 我们也在使用 LPS 时测定 IL - 3 和 IL - 4 的效果，以此排除由于在 LPS/d-GAL 制剂中使用重组蛋白质带来的可能的人为因素干扰.我们也试验了在注射 LPS 前或后使用剂量为 100 μ g/每只小鼠的 IL - 6 是否能防止内毒素死亡.

试验 2 的结果（图 2 ）与试验 1 的一致. 在此试验中，也用 IL - 6 防止小鼠由于内毒素死亡. 当 IL - 6 与 LPS 一起使用时，注射 LPS 后 24 小时的保护结果依赖于剂量：剂量为 20 、 4 、 0.8 μ g/每只小鼠时，死亡率分别为 30 % (p=0.03)、 50 % (p=0.16)、 70 % (p=0.61)，而剂量为 100 μ g/每只小鼠（死亡率 60 %， p=0.33 ）比剂量 20 μ g/每只小鼠的保护效果差. LPS 注射前或后使用 100 μ g/小鼠与相同剂量和 LPS 一起使用所观察到的保护结果相当. 在注射 LPS48 小时后的存活率与前面的相似.

在注射 LPS 时使用 IL - 4 对防止小鼠因内毒素死亡无效，而 IL - 2 会降低小鼠的存活率.

图1/13 链霉蛋白酶对聚(碳酸亚乙酯)体外物质降解的影响

(仪器 +/-扫描电子显微镜; n=3)

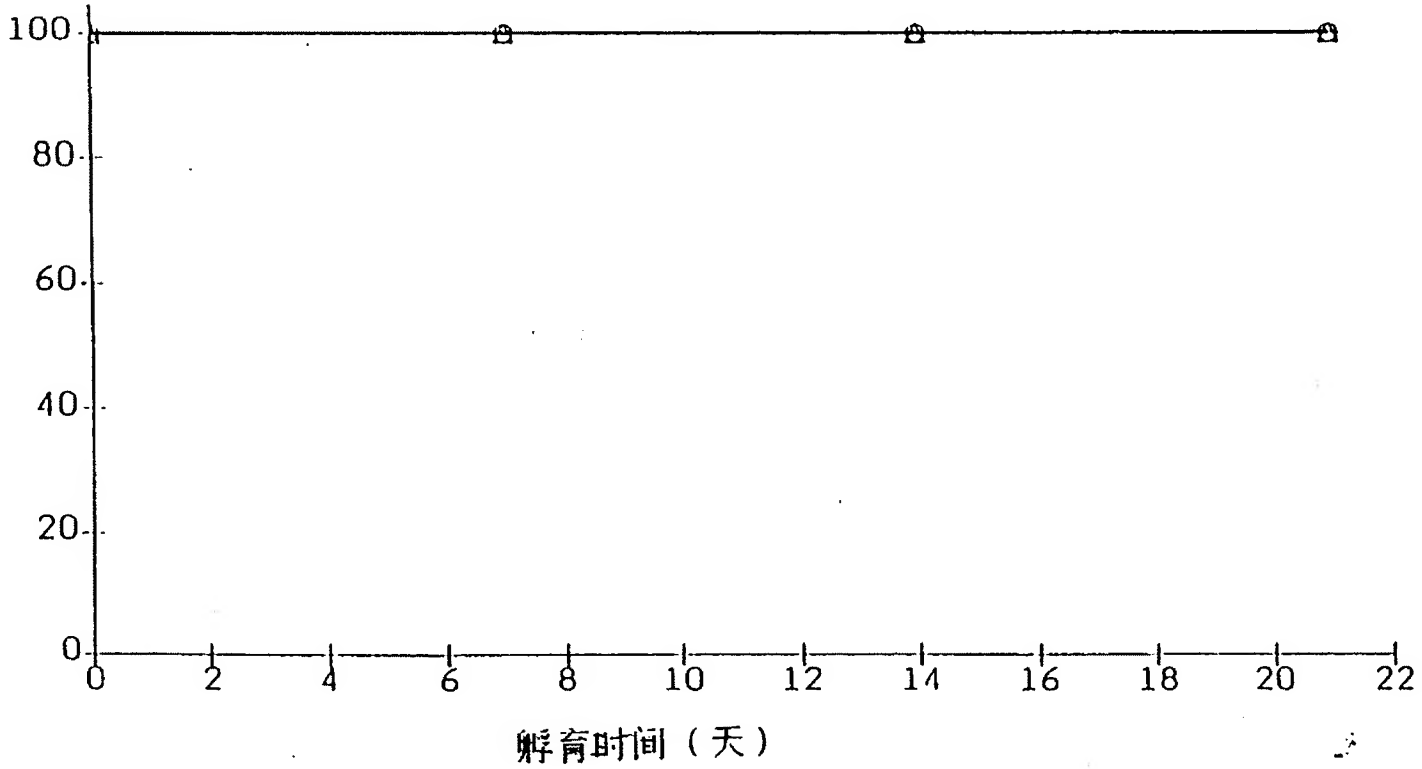


图2/13　pH 7.4磷酸生理盐水缓冲液（PBS）中聚（碳酸亚乙酯）植入剂的膨胀

（仪器 +/-扫描电子显微镜；n=3）

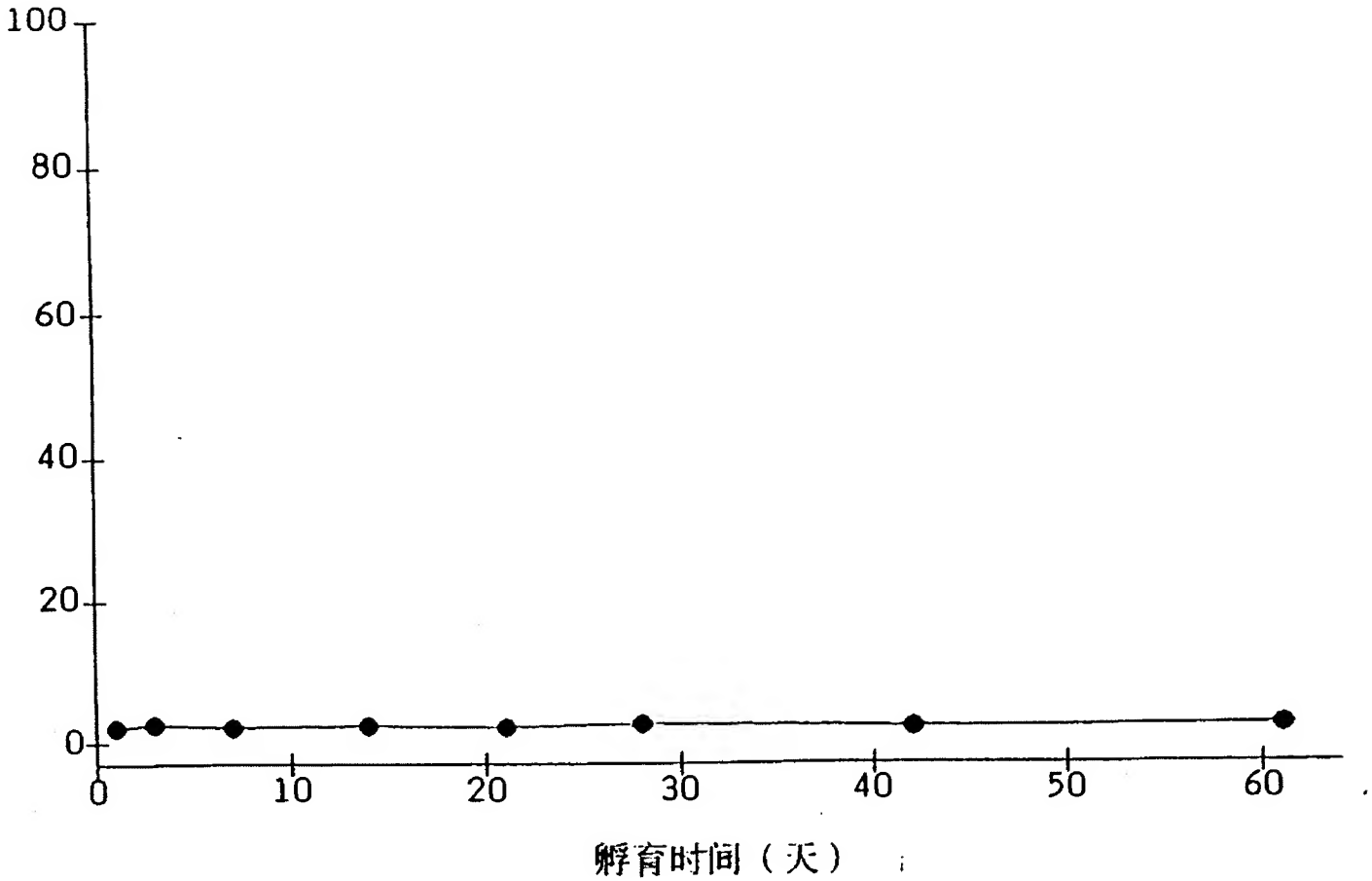


图3/13 D-葡萄糖-引发的聚(DL-乙交酯-丙交酯)共聚物(PDLLA)(54.6:45.4)和聚(碳酸亚乙酯)(PEC)的非多孔表面(仪器+扫描电子显微镜, n=3)

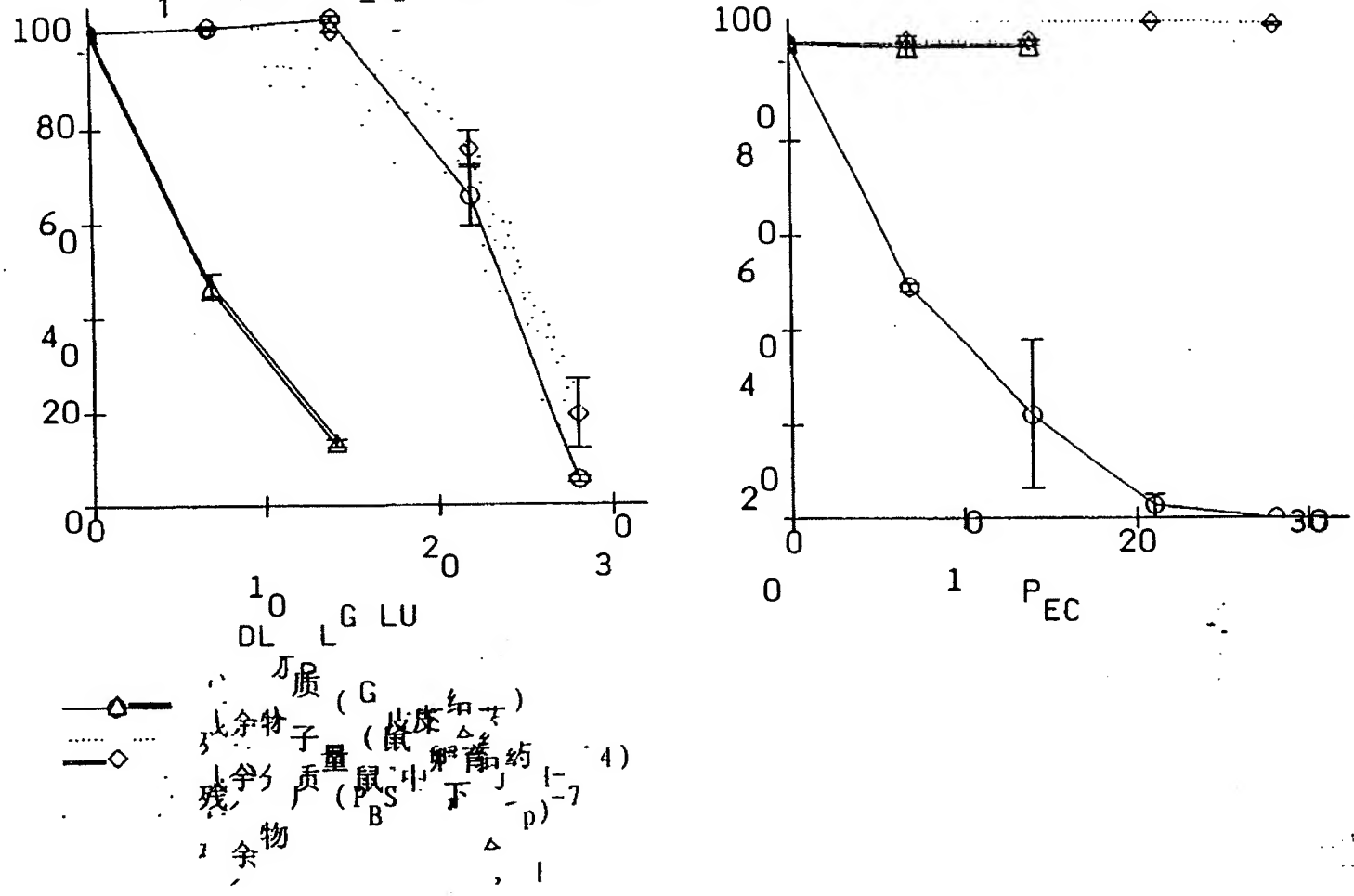


图4/13　皮下用于鼠体的聚（碳酸亚乙酯）衍生物的物质降解

（仪器 +/-扫描电子显微镜； n=3）

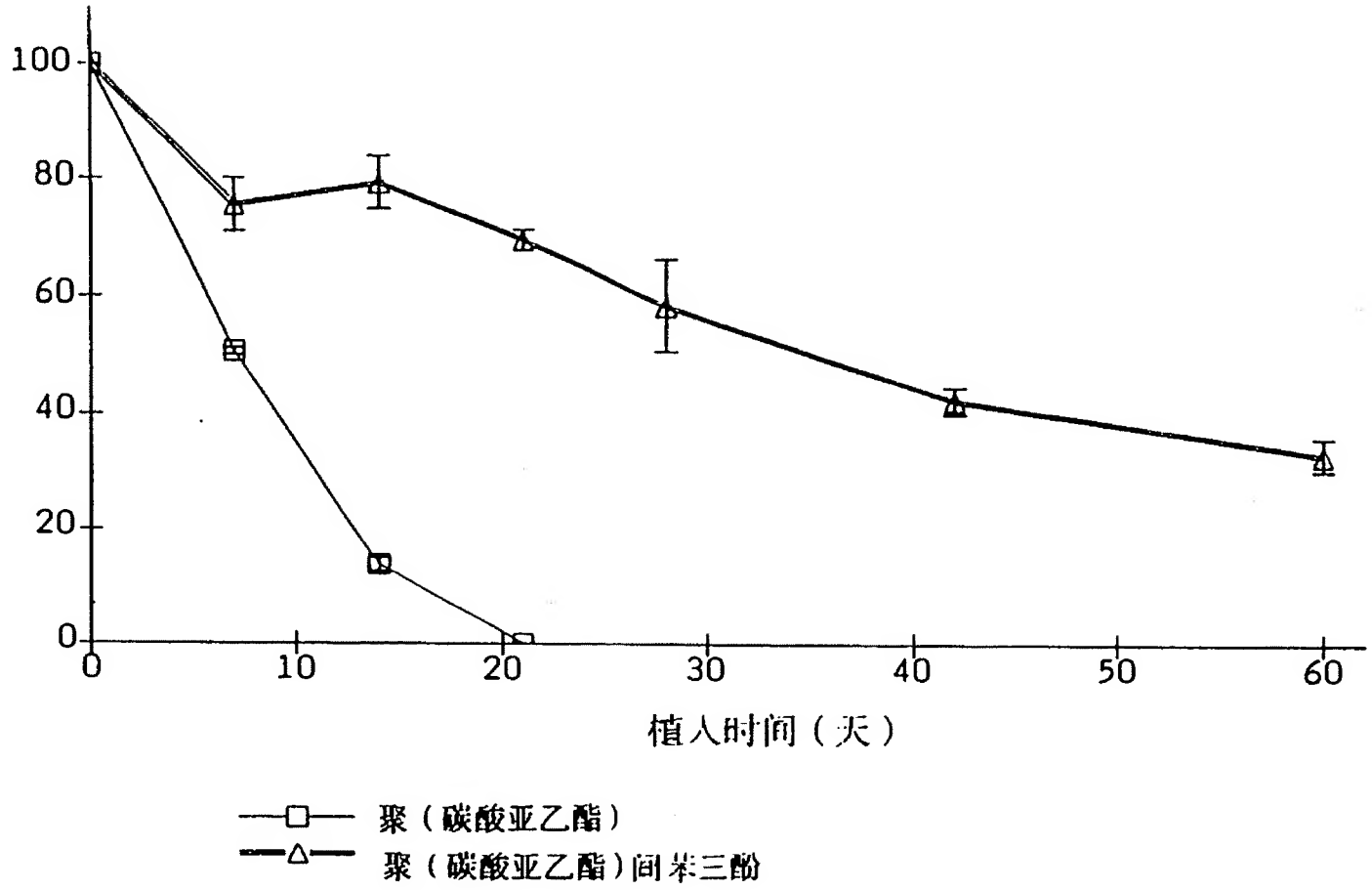


图5/13　多核细胞中聚（碳酸亚乙酯）诱发的过氧化物（细胞色素 C试验）

（仪器 +/-扫描电子显微镜；n=3）

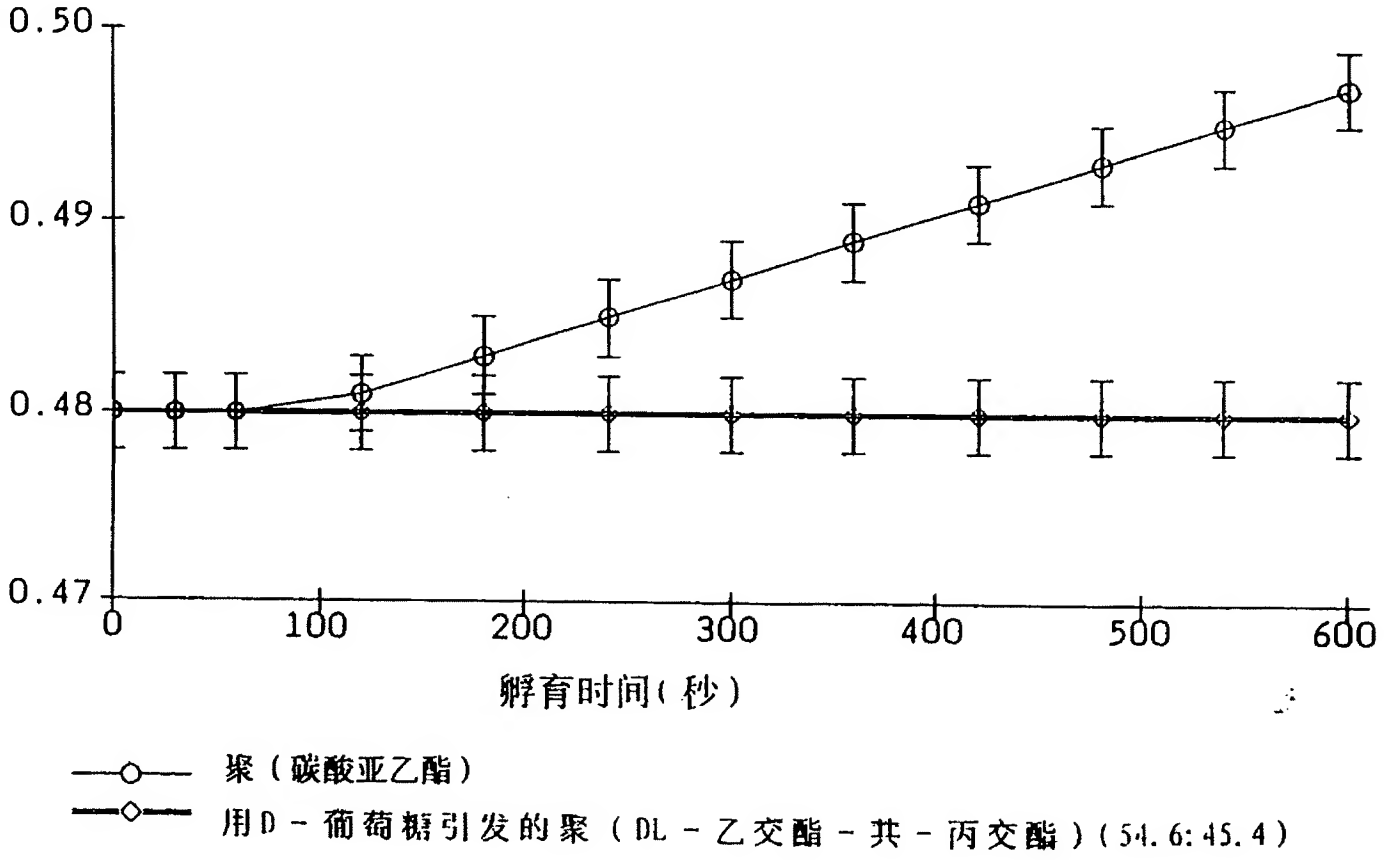


图6/13　聚（碳酸亚乙酯）体内和体外物质降解的分子量函数

（仪器 +/-扫描电子显微镜；n=2－6）

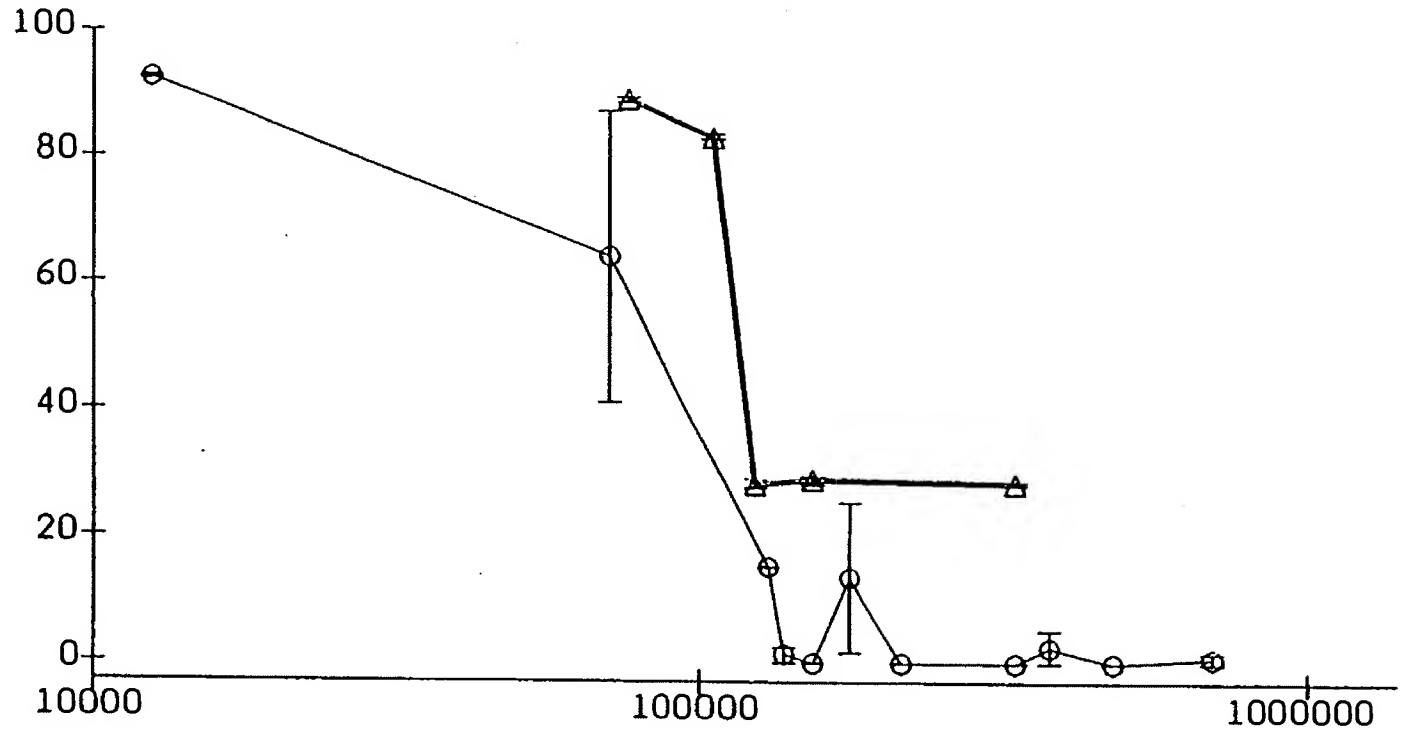


图7/13　鼠皮下用约14天后，过氧自由基清除剂对聚（碳酸亚乙酯）物质降解的影响（仪器：扫描电子显微镜，n=3）

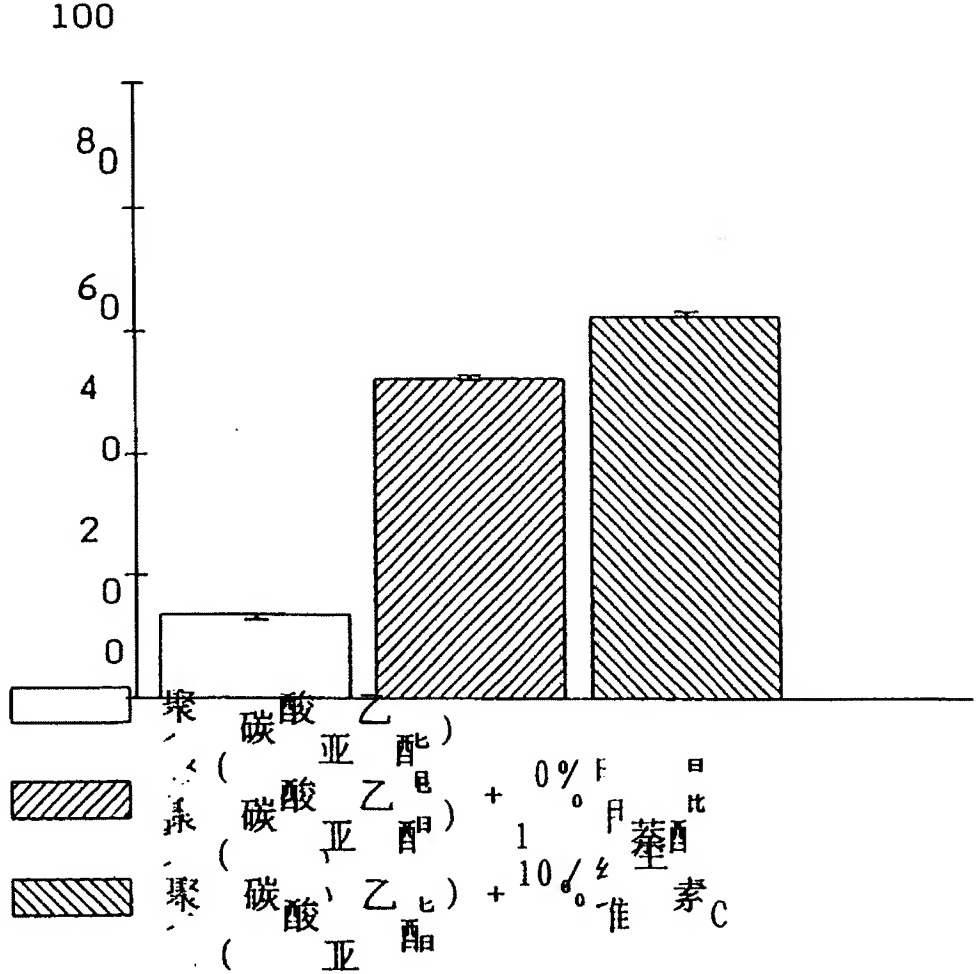
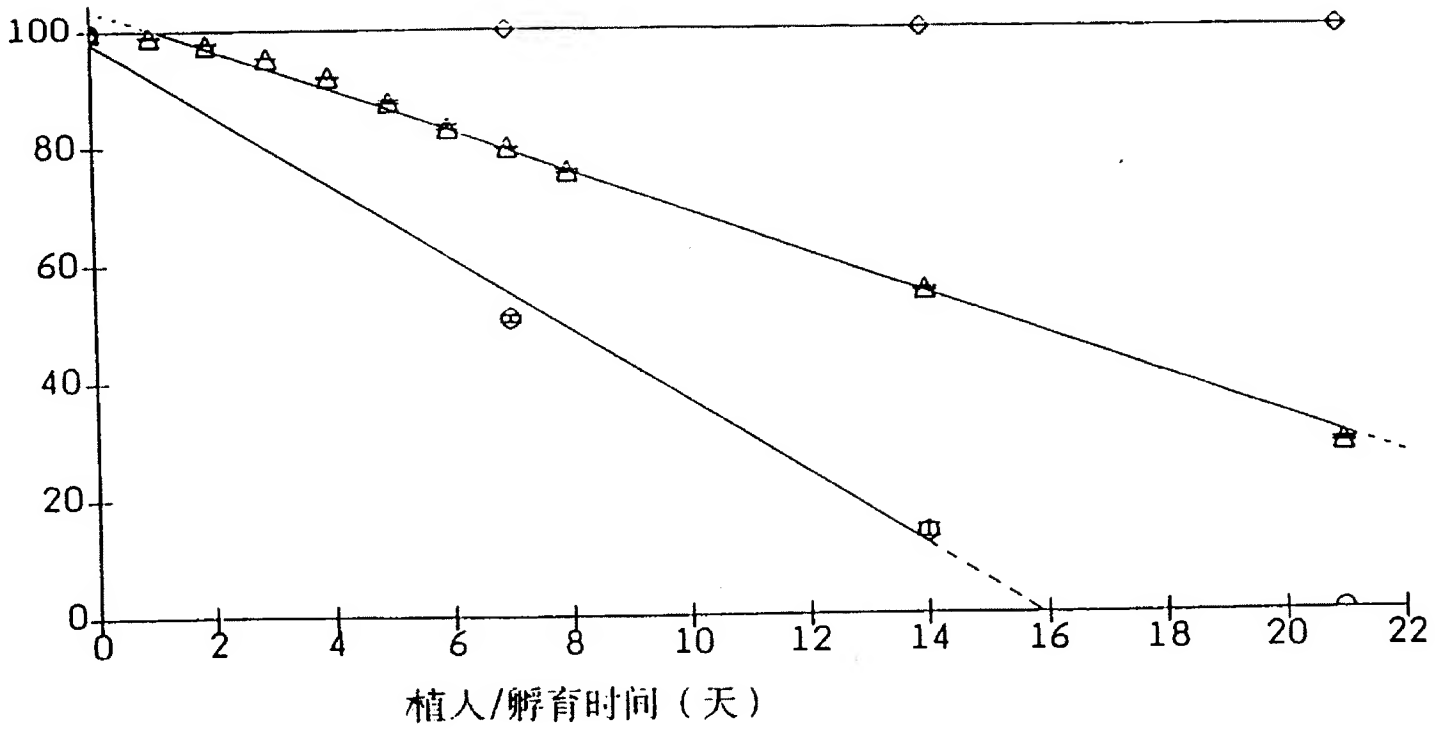


图8/13　体内及体外聚（碳酸亚乙酯）的物质降解

（仪器 +/-扫描电子显微镜；n=3）



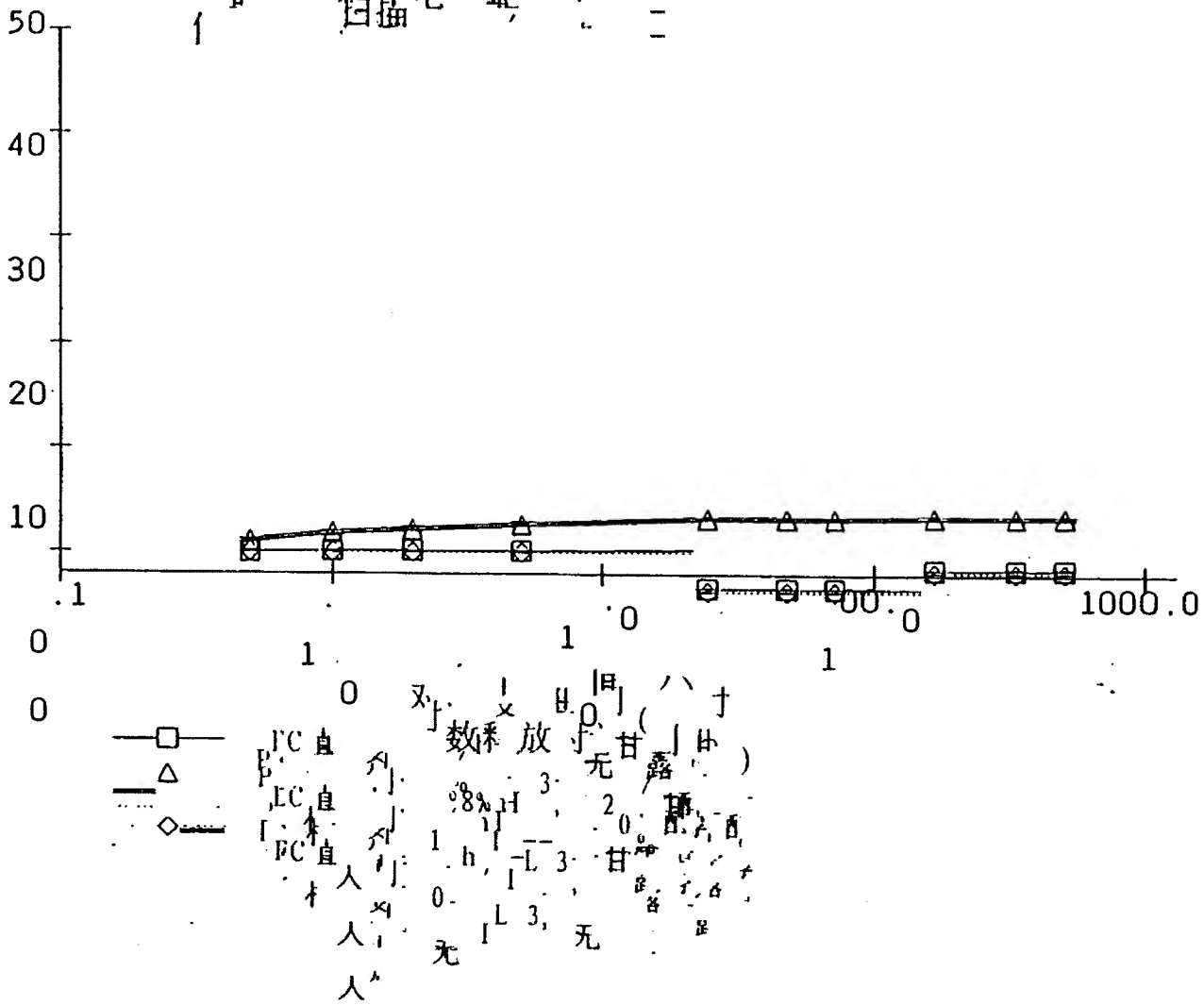
残余物质 (%)

○　鼠皮下用药

△　在过氧化钾水溶液（140mM，pH = 12）中孵育

◇　在水溶液（pH=12）中孵育

图 / 3 体 人 （碳酸 乙酯 （ ） 植入剂中
释 l L-3 血 基 hI -3 法 L ISA）
（ 器 +/- 子 镜 L ） 物 E
（ 器 扫描 电 ： 二
1



6

图10/13由鼠皮下聚（碳酸亚乙酯）植入剂中释放hIL-3（hIL--3分析法：HPLC）（仪器 +/-扫描电子显微镜；n=3）

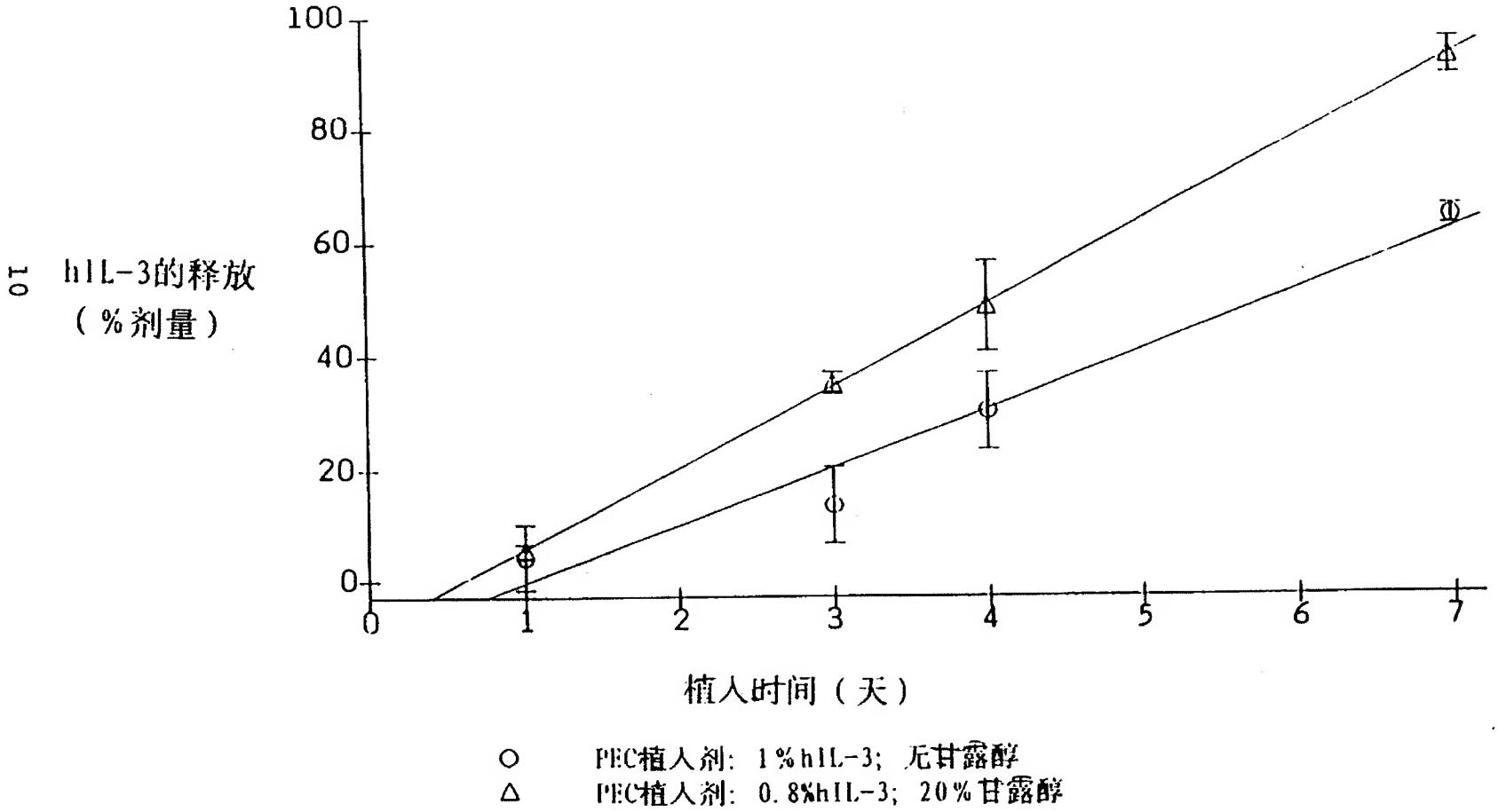


图1[illegible]

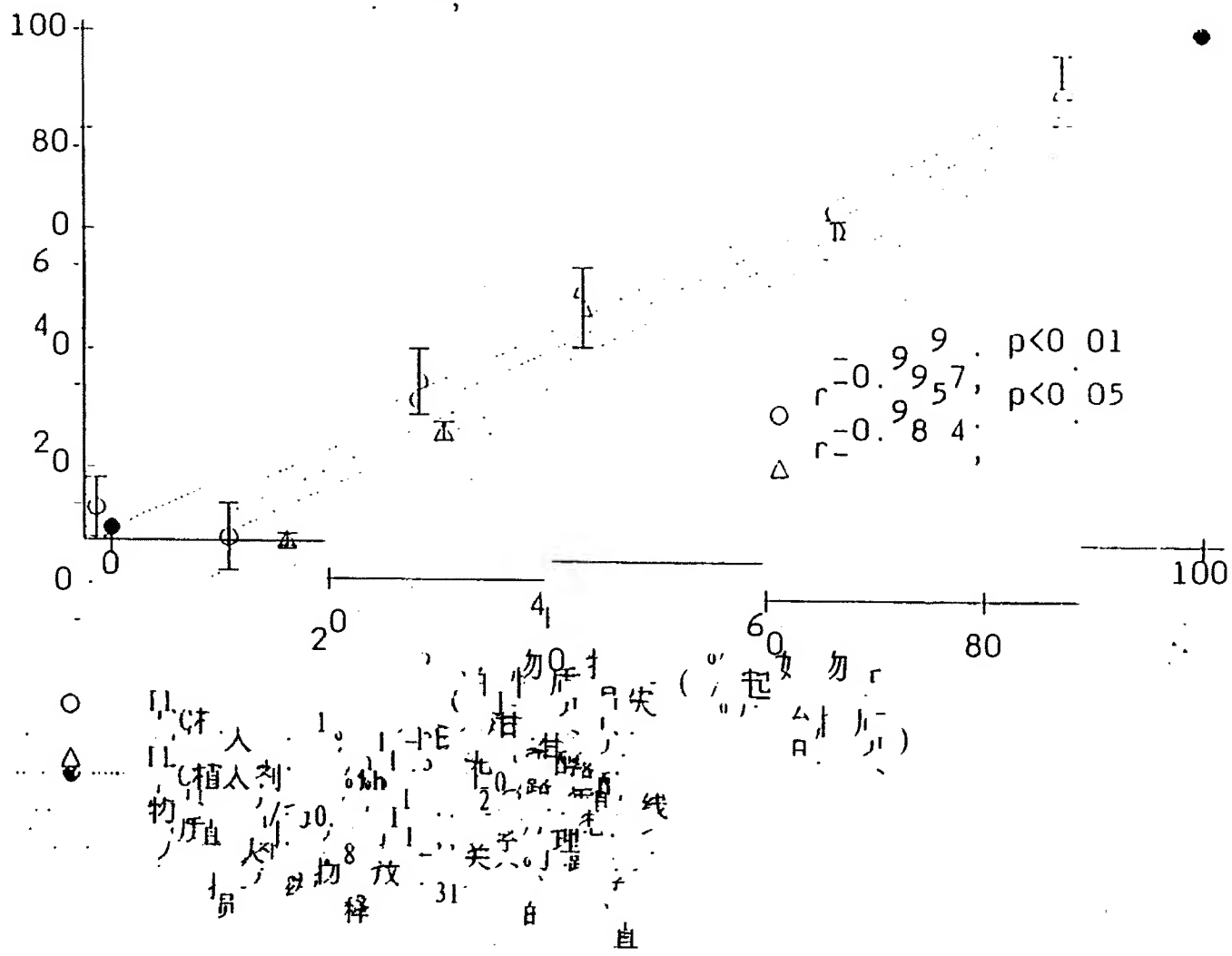

release of
hIL-3
(% of dose)
100
80
60
40
20
0
0
20
40
60
80
100
r=0.999, p<0.01
r=0.957, p<0.05
r=0.984

图12/13鼠及兔的SMS－PA血浆浓度

（仪器 +/-扫描电子显微镜; n=2－3）

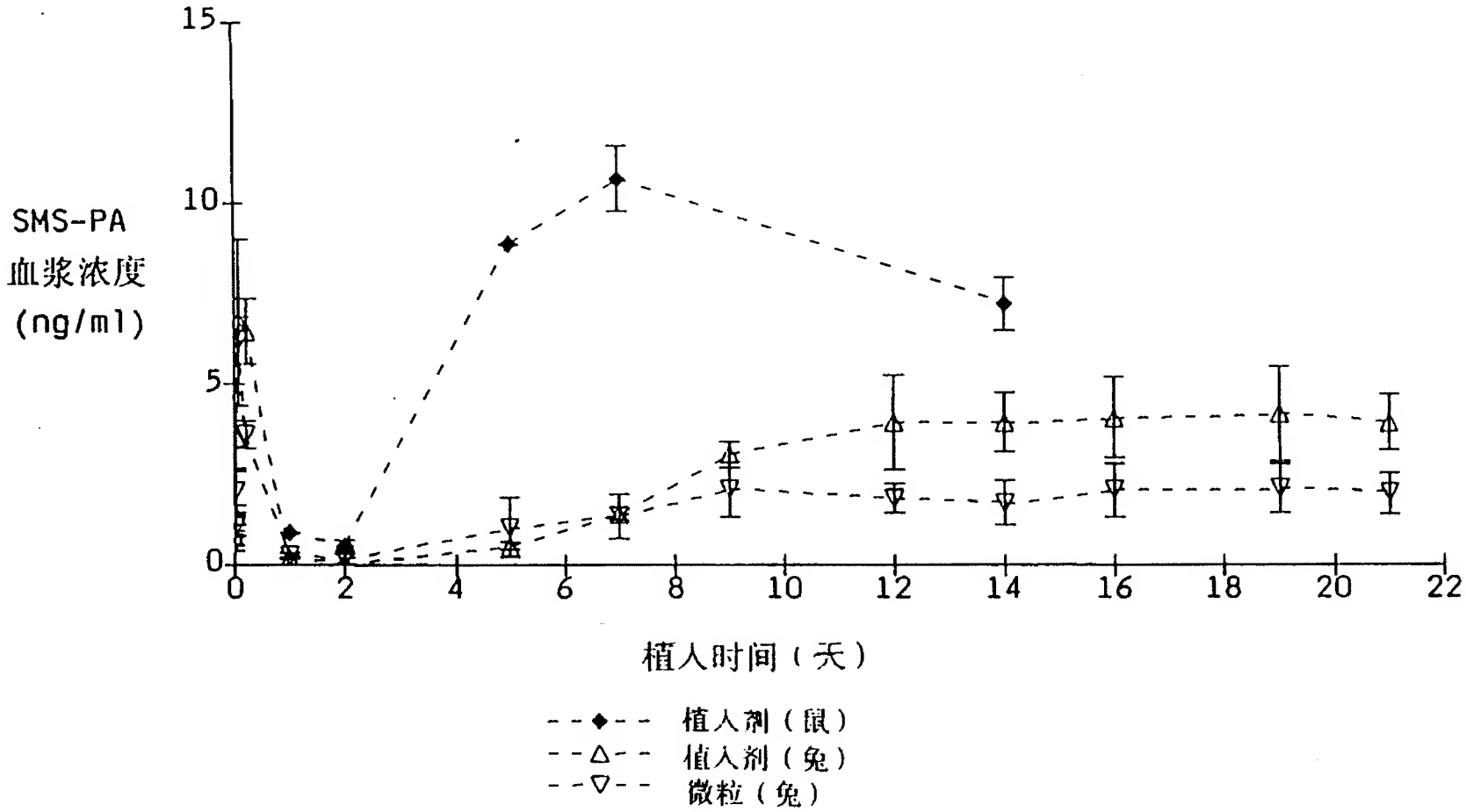
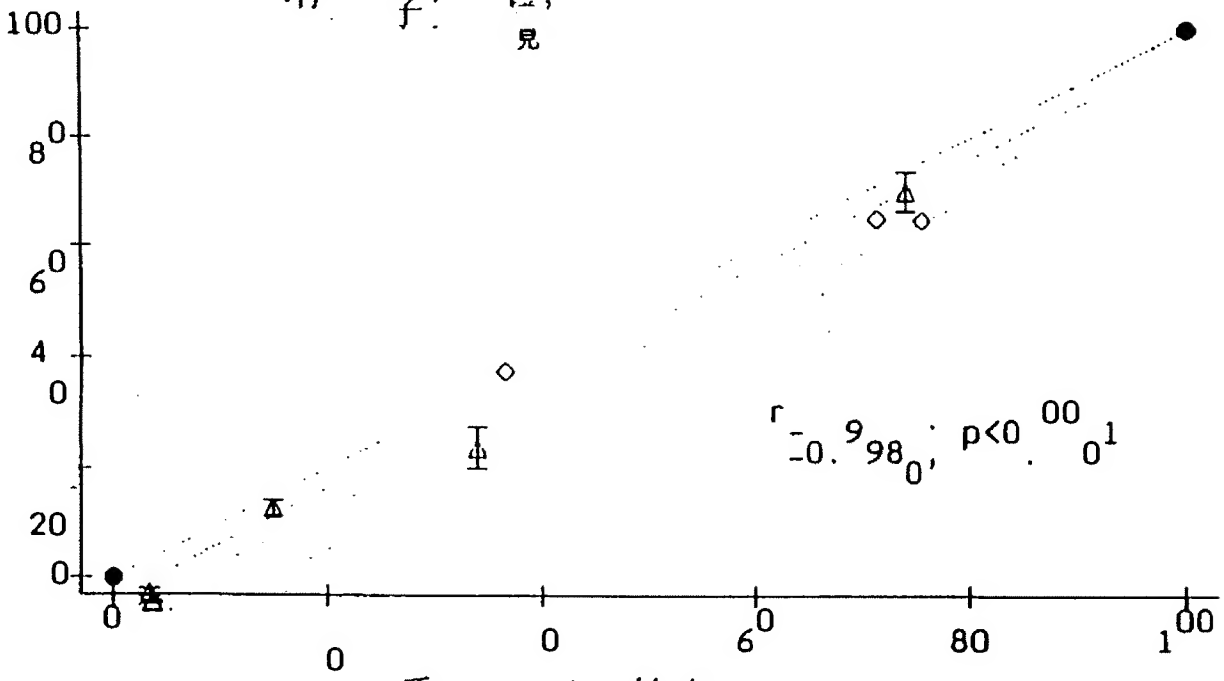


图13/13 鼠及兔的[illegible]（硫[illegible]亚乙酰）（PEC 及S[illegible]）物质[illegible]关[illegible]（皮[illegible]酸SMS-PA，分析）[illegible]HMS）[illegible]A的[illegible]用[illegible]（仪器[illegible]+/-[illegible]柱[illegible]系[illegible]，做下[illegible]n=2）[illegible]。[illegible]P（[illegible]电[illegible]量[illegible]子[illegible]钰，[illegible]见



Q物质[illegible]失4%[illegible]包[illegible]勿[illegible]质）

△ P[illegible]自[illegible]（[illegible]）

● [illegible]植[illegible]失[illegible]1[illegible]9[illegible]1[illegible]线[illegible]且[illegible]

◇ [illegible]植[illegible]S[illegible]并系[illegible]理[illegible]

[illegible]，[illegible]人[illegible]1[illegible]9[illegible]免

[illegible]S P

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(54) Title: POLYMERIC MATRICES AND THEIR USES IN PHARMACEUTICAL COMPOSITIONS

(57) Abstract

This invention provides pharmaceutical compositions comprising polymeric matrices, especially those comprising IL-6 as an active ingredient. Specific novel poly(ethylenecarbonate) polymers are also provided for more general use as matrix materials in sustained release compositions containing pharmacologically active compounds, as are methods of using of IL-6 for treatment of conditions mediated by IL-1 and/or TNFα, e.g., certain autoimmune and inflammatory conditions, as well as septic shock.

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POLYMERIC MATRICES AND THEIR USES IN PHARMACEUTICAL COMPOSITIONS

This invention relates to pharmaceutical compositions comprising polymeric matrices, especially those containing IL-6 for use in treating diseases mediated by IL-1 and/or TNFα, e.g., chronic inflammatory conditions. The specific polymers of the invention, especially the poly(ethylene carbomate) polymers described herein, however, are shown to be more generally useful as matrix materials in sustained release compositions containing pharmacologically active compounds, and in particular to have the novel, unexpected, and highly desirable property of undergoing nonhydrolytic surface erosion in vivo. Therefore, matrices comprising other drugs are also exemplified and provided, together with processes for preparing the polymers and to pharmaceutical compositions containing them. Moreover, the use of IL-6 to treat conditions mediated by IL-1 and/or TNFα is novel and unexpected (many such conditions were previously believed to be exacerbated by IL-6), thus the invention further provides a new use for IL-6 in the treatment of, e.g, chronic pathogen-induced inflammatory conditions, demyelinating diseases, and acute and hyperacute inflammatory conditions such as septic shock.

I. Treatment of diseases mediated by IL-1 and/or TNFα

Many spontaneously occuring, chronic inflammatory conditions have an unknown, (possibly autoimmune) etiology and are believed to be mediated by IL-1 and/or TNFα. For example, multiple sclerosis (MS), a crippling nerve disorder characterized by disseminated patches of demyelination in the brain and spinal cord, has occupied the attention of research organizations for many years. Although the precise etiology of multiple sclerosis is not fully

understood, it is believed to have a strong autoimmune component, as indicated, e.g., by the increased incidence of certain HLA antigens in patients having the disease. Currently available anti-inflammatory drugs such as ACTH (adrenocorticotropic hormone) or corticosteroids, e.g., prednisone, appear to hasten recovery in acute attacks, especially when administered early in the episode, but do not affect the underlying etiology of the disease. Long term administration of corticosteroids or immunosuppressants carries risks of serious side effects. A recombinant form of IFN-β_1 was recently shown to reduce short term plaque formation, but has not been shown to affect the long term progression of the disease. Evaluation of treatment efficacy is complicated by the fact that the natural progression of the disease is one of spontaneous remission and chronic relapse. In short, despite many years of intensive research, there is so far no generally accepted specific therapy for this very serious disease.

Other chronic inflammatory conditions are believed to be induced by external agents, e.g., pathogens. For example, Lyme disease is a serious chronic condition initiated by infection with the tick-born spirochete Borrelia burgdorferi. Following an initial acute phase characterized by skin lesions and flu-like symptoms, the disease progresses to a chronic phase which may be characterized by arthritis and chronic neurologic abnormalities. The disease is usually treated with antibiotics and nonsteroidal anti-inflammatory agents, but an optimal therapy, particularly for the established disease, is not yet established.

Acute or hyperacute, uncontrolled inflammatory conditions may also be caused by external agents, e.g., severe burns or severe infections. For example, septic shock, and in particular adult respiratory distress syndrome (ARDS), is a life threatening condition for which no effective treatment exists at present. Onset is rapid, and mortality generally exceeds 50%. Septic shock usually results from severe bacterial infection and is typically characterized by fever often followed by hypothermia in the later stages, fluctuating blood pressure (hyperdynamic syndrome) followed by hypotension in the later stages, metabolic

acidosis, impaired mental functioning, and widespread organ dysfunction, ultimately, in many cases, ending in death. Most commonly, septic shock results from gram-negative bacterial infection (endotoxic shock), but it may also result from gram-positive bacterial infections or other infections. The term "septic shock" as used herein is thus to be interpreted broadly to mean a shock state, including ARDS, resulting from a microbial infection, especially a bacterial infection, most especially a gram-negative bacterial infection.

IL-6 is a known cytokine. It is known to be useful in the treatment of various conditions, e.g., thrombocytopenia and certain cancers. It is produced by the body usually in response to bacterial infections and has been implicated in the mediation of inflammation, fever, and septic shock. It is a potent immunostimulant and indeed some literature suggests that IL-6 driven mechanisms cause certain autoimmune or inflammatory diseases, including systemic lupus erythematosis, multiple sclerosis, and rheumatoid arthritis, as well as septic shock.

It is thus very surprising to discover that IL-6 is useful in the treatment of chronic inflammatory diseases (other than glomerulonephritis), e.g., multiple sclerosis, and in the treatment of acute and hyperacute inflammatory conditions, e.g., septic shock. The mechanism of this action is unclear, but without intending to be bound by any particular theory, we believe that, through a feedback mechanism, IL-6 can suppress or inhibit the expression, release or function of other cytokines, particularly TNFα and/or IL-I, possibly by upregulating the release of soluble TNFα receptor and/or IL-I receptor antagonist, thereby suppressing the activity and resulting autoimmune, inflammatory, or shock conditions that are principally mediated by these cytokines. In the case of conditions characterized by IL-6 mediated complement-activating antigen-antibody (IgG) complexes, particularly glomerulonephritis (which is usually caused by aggregation of such complexes in the kidney), however, IL-6 is shown to exacerbate the condition. Thus, we have shown that IL-6 is curative in animal models for MS and Lyme arthritis, for example, which are believed to be

driven primarily by IL-I and/or TNFα, but exacerbates the glomerulonephritis in lupus mice, which is believed to be directly driven by IL-6. We have also shown that IL-6 is curative by itself in mouse models of endotoxic shock, which is likewise hypothesized to be driven principally by IL-I and/or TNFα.

IL-6 is therefore considered to be useful as an agent for suppressing or inhibiting the expression, release or function of TNFα and/or IL-I, and especially in the treatment of inflammatory conditions other than glomerulonephritis, and in the treatment of septic shock. Inflammatory conditions which may be treated using IL-6 include, for example, arthritic conditions, particularly pathogen-induced arthritic conditions, for example, Lyme disease arthritis, bacterially induced arthritis, and polioarthritis; multiple sclerosis and other demyelinating diseases (i.e., diseases characterized by demyelination in the nerves, brain, and/or spinal cord, including, e.g., multiple sclerosis, acute disseminated encephalomyelitis or postinfectuous encephalitis, optic neuromyelitis, tinnitus, diffuse cerebral sclerosis, Schilder's disease, adrenoleukodystrophy, tertiary Lyme disease, tropical spastic parapoesis, and other diseases wherein demylination, especially autoimmune-mediated demyelination, is a major symptom); acute severe inflammatory conditions such as burns, septic shock, meningitis, and pneumonia; and autoimmune diseases including polychondritis, sclerodoma, Wegener granulamatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, psoriasis, psoriatic arthritis, Steven-Johnson syndrome, idiopathic sprue, autoimmune inflammatory bowel disease (including e.g. ulcerative colitis and Crohn's disease), endocrine ophthalmopathy, Graves disease, sarcoidosis, primary billiary cirrhosis, juvenile diabetes (diabetes mellitus type I), uveitis (anterior and posterior), keratoconjunctivitis sicca and vernal keratoconjunctivitis, and interstitial lung fibrosis.

The invention thus provides:

i) A method of

inhibiting the expression, release or function of TNFα and/or IL-I;

of treating or preventing an inflammatory condition other than glomerulonephritis;

of treating or preventing a condition mediated by IL-I and/or TNFα;

of treating or preventing any of the conditions described above;

of treating or preventing a demyelinating disease, e.g., multiple sclerosis;

of treating or preventing an externally induced inflammatory condition;

of treating or preventing an inflammatory response to a severe acute infection, e.g., septic shock, meningitis, or pneumonia;

of treating burns;

of treating or preventing a chronic pathogen-induced inflammatory condition, e.g., Lyme disease;

said method comprising administering a therapeutically or prophylactically effective amount of IL-6, e.g., a TNFα and/or IL-I inhibiting amount of IL-6, e.g., rhIL-6, (e.g., especially wherein IL-6 is administered as the sole therapeutic or prophylactic agent, or optionally administered in conjunction with antimicrobial or vasoactive agents, e.g., optionally not in conjunction with TNFα agonists or antagonists or with anti-TNFα antibody); optionally in slow release or depot form, e.g., in association with a polymeric matrix, e.g., a poly(ethylene carbonate) matrix as further described herein, to a subject, e.g., a mammal, e.g., a human being, in need of such treatment or prophylaxis;

ii) The use of IL-6, e.g., rhIL-6, in the manufacture of a medicament for use in the method of (i), e.g., for treating or preventing any one of the conditions listed under (i) above, wherein the medicament is optionally in slow release form, e.g., optionally further comprising a polymeric matrix, e.g., a poly(ethylene carbonate) matrix as further described herein;

iii) The use of IL-6, e.g., rhIL-6, for the treatment or prevention of any of the conditions listed under (i) above; and

iv) A pharmaceutical composition comprising IL-6, e.g., rhIL-6, for use in the method of (i), e.g., for treating or preventing any of the conditions described in (i) above, optionally in slow release form, optionally further comprising a polymeric matrix, e.g., a poly(ethylene carbonate) matrix as further described herein; for example, a sustained release composition (i.e., a composition which biodegrades in vivo over a period of days, weeks, or months) comprising IL-6 in a polymeric matrix, e.g., in the form of a microparticle or depot, e.g., where the polymer exhibits nonhydrolytic surface erosion in vivo, especially any of the drug delivery systems described herein, for use in the treatment of any of the above-mentioned conditions, e.g., for the treatment of a chronic inflammatory condition.

By IL-6 is meant any compound corresponding to the known varieties of interleukin-6 (also known as interferon beta-2 (IFN-β_{II}), B-cell stimulatory factor 2 (BSF-2), interleukin HP-1 (HR1), hepatocyte stimulating factor (HSF), hybridoma plasmacytoma growth factor (HPGF), and 26kD factor). Recombinant IL-6 is preferred, although nonrecombinant IL-6 can also be used, e.g., as produced by IL-6 secreting cancer cell lines. IL-6 is commercially available or may be produced by known methods, e.g., as described in EPA 0 220 574, EPA 0 257 406, EPA 0 326 120, WO 88/00206, GB 2 063 882, or GB 2 217 327, the contents of which applications are incorporated herein by reference. The IL-6 may be glycosylated, e.g., as produced by eukaryotic cells, e.g., CHO cells, or nonglycosylated, e.g., as produced by prokaryotic cells, e.g., E. coli. Recombinant human IL-6 (rhIL-6) is preferred, although IL-6 is known to be active cross-species, so that IL-6 derived from nonhuman sources could

also be used and is included within the meaning of IL-6 herein. Proteins having minor variations in the sequence of IL-6, e.g., addition, deletion, or mutation of 1, 2, 3 or more amino acids; fusion proteins comprising IL-6 and another protein; active fragments of IL-6; and/or other such variant, truncated, or mutated forms of IL-6, which have IL-6 activity are intended to be encompassed within the meaning of IL-6 herein.

Suitable pharmaceutical compositions comprising IL-6, together with a pharmaceutically acceptable diluent or carrier are known. The IL-6 may be administered parentally, e.g., in the form of an injectable solution or suspension, e.g., according to or analogously to description in Remington's Pharmaceutical Science, 16th ed. (Mack Publishing Company, Easton, PA 1980). Suitable carriers include aqueous carriers such as saline solution, Ringer's solution, dextrose solution, and Hank's solution, as well as nonaqueous carriers such as fixed oils and ethyl oleate. For ordinary parental administration, the IL-6 is available in lyophilized form in unit dose amounts which may be mixed with the carrier to form a suitable solution or suspension for injection.

Alternatively, the IL-6 may be administered using an implantable or sustained release drug delivery system, e.g., in microparticle or depot form in association with a polymer, to form a polymeric matrix whereby the drug is released slowly from the matrix. This is preferred, e.g., where the condition to be treated is chronic, e.g., a chronic inflammatory condition, and the requisite treatement extends over a period of weeks or months. By polymer is meant any suitable (e.g., pharmacologically acceptable) linear, high molecular weight molecule formed of repeating units (including homopolymers, co-polymers, and heteropolymers), optionally branched or crosslinked, which may be made, e.g., by polymerization of a single molecule or from the co-polymerization of more than one molecule (e.g., poly(ethylene carbonate) from ethylene oxide and carbon dioxide as described below), and optionally containing interruptions in the polymer chain with other units. Preferably, the polymer is linear and is composed of carbon, oxygen and hydrogen, e.g.,

poly-DL-lactide-co-glycolide, polyethylene glycol, or poly(ethylene carbonate). Preferably, the polymer exhibits non-hydrolytic surface erosion, e.g., a poly(ethylene carbonate) as herein further described.

The dosage will of course vary depending on the exact type of IL-6 employed, the host, the mode of administration, and the nature and severity of the condition being treated. The IL-6 is administered to larger mammals, e.g., man, by subcutaneous injection or in sustained release form to provide a dosage of from 0.5 µg/kg/day to 30 µg/kg/day, preferably from 2.5 µg/kg/day to 10 µg/kg/day, or in any other dosage which is safe and effective for in vivo activity in known therapeutic applications of IL-6, e.g., in a platelet-increasing dosage. In the case of severe acute inflammatory conditions, e.g., septic shock, higher dosages administered i.v. may be desirable to achieve a rapid and strong response. Frequency of IL-6 administration may optionally be reduced from daily to every other day or every week, or longer in the case of sustained release forms, which are preferred when the treatment is given over longer periods of time. IL-6 treatment may result in chills, fever, and flu-like symptoms, which normally can be treated or prevented with co-administration of nonnarcotic analgesics such as aspirin, acetaminophen or indometacin. Other significant side effects ordinarily appear only at higher dosages, e.g., above 10 µg/kg/day, and can ordinarily be relieved by reducing the dosage.

II. Polymeric matrices for sustained release

The invention further provides pharmaceutical compositions suitable for sustained release of drugs, which are suitable, e.g., for administration of IL-6, e.g. in the above described indications, as well as for other drugs. The pharmaceutical compositions are especially those comprising polymers of poly(ethylene carbonate), sometimes referred to as as poly(ethylene carbonate)s or PECs.

Although the prior art provides some examples of poly(ethylene carbonate)s for use in drug delivery systems, the prior art does not disclose the particular polymers of the invention and does not disclose polymers capable of undergoing nonhydrolytic surface erosion in vivo. The prior art also does not disclose such drug delivery systems for the delivery of certain of the particular drugs disclosed herein, e.g., IL-6, nor does it suggest that a sustained release system would be desirable for delivery of such drugs.

Particularly surprising are the degradation characteristics of the polymers of the invention. On the basis of general chemical knowledge, it is expected that carbonate ester bonds are in principle cleavable. However, polycarbonates have been proved to be stable under moderate conditions in vitro.

According to Chem. Pharm. Bull. 31(4), 1400 - 1403 (1983) poly(ethylene carbonate)s are degradable in vivo, but the polymer tested was not clearly identified, e.g. by modern spectroscopical methods. According to page 1402, in vivo degradation was only explainable as due to the influence of hydrolytic enzymes.

According to Chem. Pharm. Bull. 32 (7), 2795-2802 (1984) microparticles were made of poly(ethylene carbonate) containing Dibucaine. Although the description relates to the firstly cited art, the release of Dibucaine was not seen to be related to in vitro or in vivo degradation pattern of the polymer, but to diffusion through the polymer. Also here the physical and chemical properties of the poly(ethylene carbonate) tested were not sufficiently evaluated.

According to Makromol. Chem. 183, 2085 - 2092 (1982), especially page 2086, carbon dioxide epoxide polymers are considered to be biodegradable and it is said that preliminary results confirmed the biodegradability of carbon dioxide - ethylene oxide polymers and thus their use in controlled drug release. For support of the allegation regarding

the biodegradability Jinko Zoki 3 (Suppl.), 212 (1974) was cited. In this publication it was said that poly(ethylene carbonate) belongs to the group of compounds which are most easily hydrolysed and even the enzyme pronase had no difficulty in decomposing it. This means that an enzymatic hydrolysis in vitro and in vivo would be possible, since pronase is composed of a mixture of hydrolytic enzymes. However, this comment seems very doubtful. We have subjected the poly(ethylene carbonate)s of our invention in the form of pressed disks of 5 mm diameter and 25 mg weight to 10 mg/ml pronase and 5 mM $CaCl_2.2H_2O$ in phosphate-buffered saline (PBS) of pH 7.4 and to 10 mg/ml pronase E and 5 mM $CaCl_2.2H_2O$ in phosphate-buffered saline of pH 7.4 (at 37°C) and no degradation could be observed (see Fig. 1). The pronase solution was renewed every day.

It is now surprisingly discovered that a selection of poly(ethylene carbonate)s having a special ethylene carbonate content, viscosity and glass transition temperature range, which are not degradable by hydrolysis (e.g., in the presence of hydrolytic enzymes, e.g. pronase, or under basic conditions) are nevertheless degradable in vitro and in vivo, namely and exclusively by surface erosion. The expression "surface erosion" is used in the literature, especially in relation to the hydrolytic degradation of polyanhydrides and polyortho esters, but was never clearly defined.

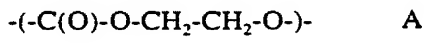
Surface erosion occurs, if there is a mass degradation merely at the surface of the polymer particles, without reduction of the molecular weight of the remaining polymer residue. Where in the literature it was alleged that surface erosion was observed, molecular weight determinations of the residual mass parallel to mass loss determinations were never carried out, and thus in fact surface erosion never was proved.

In fact, in almost all the hitherto tested polymers, polymer bulk erosion was observed. Systems exhibiting polymer bulk erosion have the significant disadvantage that if the polymer is loaded with a drug compound, e.g. a peptide, which is relatively unstable under

the influence of the biological medium to which it will be released, the drug compound is already contacted with the medium in the bulk part and can lose its activity long before it is released from the polymer. If the polymer would undergo a surface erosion, i.e. when no bulk erosion occurs, the embedded drug compound, e.g. the peptide, would remain protected from the detrimental influence of the biological medium just until the moment that the progressive surface erosion reaches the drug particles and the drug particle is released from the surface of the residual polymer mass. In case of polymer matrix drug delivery systems exhibiting surface erosion as opposed to bulk erosion, the drug particle is thus exposed to the detrimental influence of the biological medium during a shorter period of time, thereby allowing for longer, higher and more consistant release of pharmacalogically active drug from the polymer matrix.

For polyanhydrides in recent publications in Proc. Nat. Acad. Sci. USA 90, 552-556 (1993) and 90, 4176-4180 (1993) some characteristics of a surface erosion - like behaviour were described. However, the whole bulk seemed to be influenced and no molecular weight determinations were performed. Further, this erosion is of the hydrolytic type.
It was now discovered, that a selected group of poly(ethylene carbonate)s, defined below, shows, in vitro as well as in vivo, exclusively a non-hydrolytic surface erosion.

The invention provides a polymer degradable in vivo and in vitro by surface erosion which is governed by a non-hydrolytic mechanism and having ethylene carbonate units of the formula A



A

having an ethylene carbonate content of 70 to 100 Mol%, having an intrinsic viscosity of 0.4 to 4.0 dl/g, measured in chloroform at 20°C, and having a glass transition temperature of from 15 to 50°C.

The ethylene carbonate content of the polymer according to the invention is from 70 to 100 Mol%, particularly 80 - 100 %, preferably from 90-99.9%, such as from 94 to 99.9%. The intrinsic viscosity of the polymer is from 0.4 to 4.0 dl/g, measured in chloroform at 20°C. Preferably the polymer has an inherent viscosity, measured at 20°C and a concentration of 1 g/dl in chloroform of 0.4 to 3.0 dl/g.

Its glass transition temperature is from 15° to 50°C, preferably from 18° to 50°C.

In the literature poly(ethylene carbonate)s have been described having a glass transition temperature of from 5 to 17°C.

The polymers of the invention are preferably made by co-polymerization of ethylene oxide and carbon dioxide, which production process is also a part of this invention. As a consequence of this production method, the polymer contains in most cases as a co-unit the ethylene oxide unit of the formula B

$$-(-CH_2-CH_2-O-)- \qquad B$$

If the polymers of the invention are exposed to an aqueous medium, e.g. a phosphate-buffered saline of pH 7.4, practically no medium will be transported to their bulk part, e.g. as is seen from Fig. 2. Therefore no bulk erosion will occur and the remaining mass will be kept constant (100%) for a period of at least 28 days, e.g. as shown in the right graph of Fig. 3.

Poly-DL-lactide-co-glycolides are at present the most commonly used matrix materials for sustained drug release systems. Such polymers, however, unlike the polymers of the invention, are degraded by hydrolysis. For example, mass degradation in PBS as shown in the left part of Fig. 3 for one of the most sophisticated poly-DL-lactide-co-glycolide types,

namely a glucose initiated poly-DL-lactide-co-glycolide (DL-PLGGLU), described in the UK Patent GB 2 145 422.

The difference in degradation behaviour between the poly(ethylene carbonate)s of the invention and the poly-DL-lactide-co-glycolides (DL-PLG) of the art in vivo is also shown in Fig. 3. Whereas the polylactide-co-glycolide undergoes bulk erosion, as is seen from the decreasing molecular weight of the residual mass of DL-PLGGLU, the molecular weight of the residual mass of the poly(ethylene carbonate)s remains constant (100%).

The residual mass of the total implant decreases in vivo in both cases to zero within 1 month, which means that the poly(ethylene carbonate) undergoes surface erosion, rather than bulk erosion. As a consequence of the absence of bulk erosion, the loaded polymer is during storage, i.e. before its administration, impervious to moisture and remains in the same dry condition in which it has been produced. Its embedded drug, if sensitive to moisture, remains stable.

The invention also provides a process for the production of the polymer in which ethylene oxide and CO_2 are polymerized in a molar ratio of from 1:4 to 1:5 under the influence of a catalyst. It is clear that in the scope of this reaction the introduction of ethylene oxide units in the polymer chain is possible, if two epoxide molecules react with each other without intervention of a CO_2 molecule, i.e. if an oxy anion intermediate attacks another ethylene oxide molecule before being carboxylated by CO_2. It is thus probable that the polymer contains several ethylene oxide units. The polymer of the invention, if containing ethylene oxide units, has a random distribution of ethylene carbonate and ethylene oxide units according to the sum formula A_m-B_n =

$$-(C(0)-0-CH_2-CH_2-0-)-_m-(-CH_2-CH_2-0-)-_n$$

in which

$\frac{m}{n+m} \times 100 = 70$ to 100.

However, most of the ethylene oxide units in the polymers of the invention have, statistically, adjacent ethylene carbonate units, especially in those cases, in which the molar ratio of ethylene oxide units is small. That means that in these cases most of the resulting ether functions are distributed randomly between carbonate functions along the polymer chain. ^{1}H-NMR spectra of the products of the invention in $CDCl_3$ confirm this assumption. They exhibit signals at δ = ca. 4.37 ppm (Integral Ia) of the ethylene carbonate units (ethylene units between two carbonate functions), at ca. 4.29 and 3.73 ppm (Integrals Ib and Ic) of ethylene units between one carbonate and one ether function, and at ca. 3.65 ppm (Integral Id) of ethylene units between two ether functions. The proportion of ethylene carbonate units (A) is then calculated within NMR accuracy limits according to the formula:

$$\text{Mol \% of ethylene carbonate units (A):} \quad \frac{Ia}{Ia + Ib + Ic + Id} \cdot 100$$

As a structural feature of poly(ethylene carbonate)s, in the literature often their content of ether functions, instead of their ethylene carbonate content is given. The ratio of ether functions (E) in the polymers of the invention may be calculated according to the formula:

$$\text{Mol \% of ether functions (E)} = \frac{Ic + Id}{Ia + Ib + Ic + Id} \cdot 100$$

According to the PCT-Patent Application WO 92/22600 poly(ethylene carbonate)s are prepared which contain ethylene oxide units and ethylene carbonate units in a molar ratio of

2 to 400 : 2, which means that the polymer contains at least 50 Mol% of ethylene oxide and thus less than 50 Mol% of ethylene carbonate units. The application mentions the biodegradability of the polymers and their use as bioerodible matrices for the sustained release of pharmacologically active compounds. However, no data have been given that the polymers are indeed biodegradable. Generally, poly(ethylene carbonate)s having such large numbers of ether functions are scarcely biodegradable. The application does not mention any hint as to the possibility of surface erosion of the polymers.

In the Examples of the US-Patent 3 248 415 low molecular weight poly(ethylene carbonate)s of Mw = 700 - 5000 are described having less than 70 Mol% of ethylene carbonate units, different from the polymers of the invention and nothing has been mentioned about their biodegradability.

According to the PCT-Application WO 89/05664 poly(ethylene carbonate)s are described which contain in the described structure II ethylene oxide and ethylene carbonate units in a molar ratio of 1 to 8 : 1, which means that the polymer contains at least 50 Mol% of ethylene oxide and thus at most 50 Mol% of ethylene carbonate units, different from the polymers of the invention. Although, the polymers are described as to be used for biodegradable medical devices, e.g. implants which may contain a drug compound, no information have been given about surface erosion.

In the process of the invention the ethylene oxide unit content and thus the content of ether functions, which delays or inhibits the biodegradation speed of the polymer, is reduced considerably by specifying the reaction conditions such as the described molar ratio's of the reaction components, the reaction temperature and further by choosing an appropriate catalyst, e.g. such prepared from Zn $(C_2H_5)_2$ and water or acetone or a di- or a triphenol e.g. phloroglucin, in a molar ratio of from 0.9:1 to 1:0.9 or 2:1 to 1:2 respectively, or preferably

prepared from $Zn(C_2H_5)_2$ and a diol, especially ethylene glycol, in a molar ratio of from 0.9:1 to 1:0.9.

The process is preferably carried out in a solvent or dispersing agent system of an organic solvent, e.g. dioxane and CO_2. CO_2 is preferably applied in liquid form and is present in an excess. The pressure is preferably from 20 to 70 bar and the temperature preferably from 10 to 80°C, especially from 20 to 70°C.

The polymers of the invention thus obtained comprise usually less than 15% of ether functions, preferably less than 10%, particularly less than 5%, e.g. less than 3%.
The poly(ethylene carbonate)s of the invention, if prepared using the catalyst from ethylene glycol or acetone and diethylzinc exhibit low polydispersities (Mw/Mn), usually less than 5, such as less than 2.5.

In the process according to the invention the catalyst or a part of it is considered to be the chain initiator for the (co)-polymer. When the reaction is finished and the chain is complete, its final terminal group is a hydroxyl group. The opposite site of the chain, there were the chain was started up, may be occupied by the catalyst group or a fragment of it. If the catalyst is prepared from ethylene glycol and diethylzinc or water and diethylzinc, both ends of a polymer chain are supposed to be identical. However, if the catalyst is prepared from a di- or triphenol and diethylzinc, the aromatic group will be incorporated into the end of a chain, where the chain starts up, whereas the other end of the chain will be a hydroxyl group. From Fig. 4 it is seen, that poly(ethylene carbonate), if one of its terminal groups is blocked, e.g. by an aromatic initiator such as phloroglucin, is slower biodegradable. For that reason, it is assumed, that the polymer chain degradation starts at the terminal hydroxyl group(s). Alternatively, a later derivatization of a terminal hydroxyl group may also be considered, e.g. by esterification, to block terminal hydroxyl groups and to control the biodegradation of the poly(ethylenecarbonate)s of the invention. Suitable terminal ester

groups are biocompatible ester groups, like (C_{1-48}) fatty acid ester groups, preferably (C_{1-30}), especially (C_{1-18}) fatty acid ester groups, e.g. the ester groups of acetic acid and stearic acid, or a carbonic acid ester group e.g. the ethylene carbonate group, or the pamoic acid ester group or a lactic or glycolic or polylactic or polyglycolic or polylactic-co-glycolic acid ester group.

The poly(ethylene carbonate)s of the invention are stable for several hours in hot water (90-100°C) without considerable molecular weight reduction. A significant increase of the glass transition temperature is observed after exposure to boiling bidistilled water during 5 hours, e.g. up to above 18°C, e.g 28°C. By performing this reaction step, a higher polymer purity is attained. We have found that polymers treated in this manner are also better processable.

The poly(ethylene carbonate) portion of the polymers of the invention is, as said before, not hydrolysable, that is to say during at least 1 month by hydrolytic enzymes under physiological conditions or by water at pH 12 and 37°C (see Fig. 1 and 8). However, it was discovered that the polymers of the invention degrade in vivo and in vitro by surface erosion under the influence of the superoxide radical anion $O_2^{\cdot-}$. Superoxide radical anions $O_2^{\cdot-}$ are generated in inflammatory cells in vivo and ex vivo in the presence of the poly(ethylene carbonate)s of the invention as is seen from Fig. 5. Polylactide-co-glycolides, the most commonly used matrix materials for sustained drug release systems nowadays and degraded by bulk hydrolysis, do not induce the generation of superoxide radical anions $O_2^{\cdot-}$, which is shown in the same figure for the glucose initiated poly-DL-lactide-co-glycolide, which was also used for Fig. 3.

In vitro, an aqueous system was established, containing potassium superoxide as source of $O_2^{\cdot-}$ and showing surface erosion of the poly(ethylene carbonate)s of the invention

(see Fig. 8). In vitro a pH 12 was chosen, since the $0_2^{\cdot-}$ radical is sufficient stable at this pH value.

Interestingly, poly(propylene carbonate), different from poly(ethylene carbonate) by substitution of a hydrogen of the ethylene unit by a methyl group, is not at all biodegradable, as shown by Japanese authors in Chem. Pharm. Bull 31(4), 1400-1403 (1983).

Using microparticle suspensions of poly(ethylene carbonate)s of the invention a toxicological study was conducted in 48 rats for 21 days and in 24 dogs for 35 days. Two applications were done in each species at day 1 and day 17. After subcutaneous and intramuscular application of 10 and 40 mg of polymer microparticles /kg body weight no clinical signs of systemic toxicity, no relevant effects on hematological parameters, on parameters of clinical blood chemistry, on body weight, and on food consumption were observed. The application sites were tested for histophathological changes 4 and 21 days after application in rats, and 18 and 35 days after application in dogs. Besides the expected inflammation reaction no unusual histophathological changes were found.

The degradation rate of the polymers of the invention may be adjusted within wide limits, depending on their molecular weight, their ethylene oxide content, the identity of the terminal groups, e.g. biocompatible ester groups, and the presence of $0_2^{\cdot-}$ radical scavengers, e.g. vitamin C, and may last between 5 days and 6 months or longer, e.g. up to 1 year. A radical scavenger may preferably be embedded in the polymer as an additive.

The molecular weight Mw of the (co)-polymers of the invention is from 80,000, preferably from 100,000, particularly from 200,000 to 2,000,000 Daltons, determined by gel permeation chromatography with methylene chloride as the eluant and polystyrene as the reference.

Chem. Pharm. Bull. 32 (7) 2795-2802 (1984), discussed above, mentions that poly(ethylene carbonate)s having a molecular weight of from 50,000 to 150,000 Daltons were used. We have found that an in vitro and in vivo degradation of the polymer may only be attained in a satisfactory proportion when the molecular weight is above 80,000, preferably above 100,000 (Fig. 6); this is a preferred aspect of the invention.

The polymers of the invention may be used in pharmaceutical compositions, especially as matrix materials for embedding pharmacologically active compounds. Since under in vitro and in vivo conditions no bulk erosion occurs and the active compound is protected by the polymer, the active compound is released as soon as (and not before) it appears at the matrix surface due to surface erosion of the matrix. In an aqueous system in vitro at pH 7.4 containing no $O_2^{\cdot}$, only traces of active compound were released (see Fig. 9).

A further advantage of surface erosion is that the size of the pharmacologically active compound molecule does not influence its release rate.

The invention therefore provides a pharmaceutical composition of a pharmacologically active compound in a polymer, showing non-hydrolytic surface erosion, especially with a linear, especially a 1:1 linear correlation of active compound release and non-hydrolytic polymer mass degradation and active compound protection in the polymer matrix.

The compositions are preferably used in the form of microparticles or of implants.

The preparation of the pharmaceutical forms according to the invention may be carried out by methods known per se, the microparticles by appropriate spray drying or emulsifying techniques, the implants by mixing the drug compound and the poly(ethylene carbonate)s both in particulated, solid state at higher temperatures at which the poly(ethylene carbonate)s become soft and thus processable, optionally followed by cooling the mixture to solid state

and modelling it to a suitable shape. It is also possible to mix the drug compound in dissolved or dispersed state with a solution of the poly(ethylene carbonate) and to evaporate the solvent, after which the solid residue is shaped to suitable implant forms.

Pharmaceutical compositions containing microparticles may be made by working them up with suitable galenical excipients and optionally bringing them in appropriate dispensers.

Depending on the drug properties and the production process the drug loading content can vary between wide limits, in the order of 0.001 to about 70%, e.g. 0.001 to 20%, preferably of 0.001 to 5% of weight. Percolation of medium into the polymer due to a high drug loading should be avoided and restricts the upper value of the loading content.

In the medical practice of administering drug compounds every type of pharmacologically active compound may be used in combination with the poly(ethylene carbonate) of the invention. In the case of microparticles preferably those types of drug compounds are used, which are pharmacologically active in low amounts and need to have an uninterrupted blood level during extended periods, e.g. hormones, peptides or proteins, e.g. somatostatins, interferons, or interleukins, but in particular those that are unstable and will desintegrate after oral use in the gastro-intestinal system and thus preferably are administered parenterally.

The depot formulation according to the invention may be used to administer a wide variety of classes of active agents, e.g. pharmacologically active agents such as contraceptives, sedatives, steroids, sulphonamides, vaccines, vitamines, anti-migraine drugs, enzymes, bronchodilators, cardiovascular drugs, analgesics, antibiotics, antigens, anti-convulsive drugs, anti-inflammatory drugs, anti-parkinson drugs, prolactin secretion inhibitors, anti-asthmatic drugs, geriatics and anti-malarial drugs. The active agent may be

chosen from a wide variety of chemical compounds, e.g. lipophilic and/or hydrophilic active agents, including peptides, such as octreotide (described in the UK patent GB 2 234 896 A).

The active proteins or peptides are preferably cytokines, e.g. interleukins, G-CSF, M-CSF, GM-CSF or LIF, interferons, erythropoetins, cyclosporins, or hormones, or their analogues, e.g. octreotide.

The pharmaceutical compositions may be used for

immunomodulation wherein the active ingredient comprisies a cytokine, e.g. an interleukin (IL-3, IL-6), or hematopoietic colony stimulating factors (G-CSF e.g. Filgrastim, GM-CSF, e.g. Molgramostim, Sargramostim, M-CSF), e.g. as a vaccine adjuvant

achieving hematopoietic reconstitution after myelosuppresive therapy or after bone marrow transplantation, wherein the active ingredient comprises a hematopoetic growth factor, e.g. GM-CSF, G-CSF, IL-3, IL-6, Leukemia Inhibitory Factor (LIF), Stem Cell Factor (SCF), or combinations thereof

achieving high local concentration of active ingredient, e.g., wherein the active ingredient comprises a drug or cytokine, GM-CSF, IL-6, IL-2, IL-4 or combinations thereof, to stimulate protective immune response, e.g., when administered together with irradiated tumor cells or vaccine antigens (an analogy to irradiated tumor cells transfected with the respective cytokine genes)

inducing potent immune responses wherein the active ingredient comprises, e.g., GM-CSF adminstered in combination with antigens, especially tumor antigens, viral antigens or bacterial antigens

inducing wound healing with local injection of the compositions, e.g., wherein the active ingredient comprises GM-CSF

inducing Ag specific immune tolerance wherein the active ingredient is, e.g., GM-CSF combined with inhibitors of accessory molecules (co-receptors), especially inhibitors for CD28-B7 interaction, for CD40-CD40 ligand interaction, for adhesion factor interactions

accompanying therapy with a cytostatic treatment, or as a vaccine adjuvant, wherein the active ingredient is e.g. a cytokine, especially an interleukin (IL-3, IL-6) or cytokine secretion inducer, e.g. a lipid derivative, e.g. the compound described in EP 0309411, especially in Example 1, also known as MRL 953

specific immune suppression, e.g. wherein the active ingredient is an immunophilin-binding immunosuppressant, e.g., a cyclosporin (e.g., Cyclosporin A), an ascomycin (e.g., FK506), or a rapamycin (e.g. rapamycin or derivative as described in WO 94/09010, e.g., 40-O-hydroxyethyl-rapamycin)

treatment or prophylaxis of autoimmune diseases and inflammatory conditions by slow release of anti-inflammatory cytokines, e.g., IL-6, IL-10, or TGFβ; or interferons, e.g., IFN-β_1 or Betaseron; or soluble cytokine receptors or cytokine receptor antagonists e.g., for IL-I, TNFα, or IL-4

treatment or prophylaxis of allergic diseases by slow release of the soluble α-chain of the high affinity receptor for IgE (Fc_E RI)

cancer treatment, e.g. with octreotide, cytokines especially interleukins,

selective targeting e.g. for the treatment of leishmaniasis, of fungus infections, of enzyme storage illnesses (Tay Sachs, Gauckerillness),

AIDS or ARC therapy,

vaccination e.g. with tetanus toxoid vaccine

hematapoesis, e.g., wherein the active ingredient is erythropoetin

intraarticular injection into inflamed joints wherein the active ingredient is an antiinflammatory drug, especially one which are not orally bioavailable or has a very short half life e.g. IL-1β converting enzyme inhibitors, metalloprotease inhibitors.

A method for enhancing an immune response of a mammal to a vaccine comprising administering to a mammal in need of vaccination an effective amount of GM-CSF in conjunction with a vaccine has been described in the international PCT-application WO 94/01133. However, the GM-CSF was not carefully retarded in the manner according to the instant invention, which gives a nearly constant release of the active compound over a longer period of time by which the times of repeated administration of GM-CSF can be diminished.

The invention especially provides a pharmaceutical composition of a pharmacologically active compound in a polymer showing non-hydrolytic surface erosion for parenteral administration of an interleukin or CSF, particularly in a polymer as defined hereinbefore.

The invention also provides a method of administration such a composition to a subject which comprises administering it parenterally to a subject in need of such treatment.

The depot formulations according to the invention may be used for the known indications of the particular drug compound incorporated therein.

The exact amounts of drug compound and of the depot formulation to be administered depends on a number of factors, e.g. the condition to be treated, the desired duration of treatment, the rate of release of drug compound and the degradability of the poly(ethylene carbonate).

The desired formulations may be produced in known manner. The amount of the pharmacologically active agent required and the release rate thereof may be determined on the basis of known in vitro or in vivo techniques, e.g. how long a particular active agent concentration in the blood plasma remains at an acceptable level. The degradability of the matrix may also be obtained by in vitro or especially in vivo techniques, for example wherein the amount of matrix materials in the subcutaneous tissue is determined after particular time periods.

The depot formulations of the invention may be administered in the form of e.g. microparticles by oral, nasal or pulmonal, preferably subcutaneous, intramuscularly or intraveneous administration, particularly as a suspension in a suitable liquid carrier or in the form of implants, e.g. subcutaneously.

Repeated administration of the depot formulations of the invention may be effected when the polymer matrix has sufficiently been degraded, e.g. after 1, 2 or 3 weeks or 1 month.

An advantage of the poly(ethylene carbonate) matrices of the invention is that during the release of the drug compound the polymer chains are degraded to parts of a small molecular size, which are transported by the body fluids from the site of administration.

Examples of drug loadings for the preferred compound octreotide are for acromegaly, in a parenteral liquid depot formulation having microparticles which contain the peptide in an amount from at least 0.1 preferably 0.5 to 20 percent by weight relative to the (co)-polymer matrix, preferably 2.0 to 10, especially 3 to 6% of weight. The total dose of octreotide is 20 to 30 mg in acromegaly and up to 100 to 200 mg in breast cancer, e.g. for 1 month of treatment.

The release time of the peptide from the microparticles may be from 5 days to about 2 weeks or longer.

Conveniently the sustained release formulation comprises the octreotide in the (co)-polymer carrier, which, when administered to a rabbit or a rat subcutaneously at a dosage of 2 mg of octreotide per kg of animal body weight, exhibits a concentration of octreotide in the blood plasma of at least 0.3 ng/ml and preferably less than 20 ng/ml during a longer period.

The pharmaceutical compositions of the invention may contain further additives, preferably also embedded in the (co)-polymer e.g. a radical scavenger, especially as scavenger of the superoxide radical anion $O_2^{\cdot-}$. The presence of such a scavenger, e.g. menadione or vitamin C, decreases the degradation rate of the poly(ethylene carbonate) (Fig. 7).

Another type of additive is a scavenger of the hydroxyl radical, possibly developed under the influence of the superoxide radical anion $O_2^{\cdot-}$, e.g. a polyol, especially a sugar alcohol, particularly mannitol. This additive was found to have also a favourable influence on body weight gain of test animals to which e.g. microencapsulated IL-3 is administered. Without this additive the body weight gain was delayed. When the composition is in the form of microparticles the same additive or another may be added externally to the existing

microparticles, since it then has a favourable influence on the stability of a microparticle suspension - against flocculation and precipitation.

If an additive is present, then preferably in an amount of 1 to 90% of weight, related to the total weight of the formulation.

The favourable in vitro and in vivo mass degradation under the influence of the superoxide radical anion $O_2^{\cdot-}$ may be seen from Fig. 8. The degradation curves for the residual mass are approximately linear and have a different slope, since the degradation conditions in vivo and in vitro are different. The amount of degraded mass per time unit is almost constant.

The curves for the in vivo release of a pharmacologically active compound, e.g. human IL-3, under the influence of the superoxide radical anion $O_2^{\cdot-}$ are, like the degradation curves approximately linear (Fig. 10), which means that also the amount of released drug compound per time unit is almost constant.

A combination of both in vivo human IL-3 release and in vivo mass degradation was recorded in Fig. 11, showing an 1:1 correlation between in vivo mass degradation and drug release.

EXAMPLES 1-5: General procedure for the synthesis of poly(ethylene carbonate)s with a catalyst prepared from diethylzinc and water

For amount of reactants, solvent, catalyst etc. for a particular experiment see table 1.

200 ml dry dioxane and 19.5 g (158 mmole) $Zn(C_2H_5)_2$ were placed in a 750 ml flask under a N_2-atmosphere. The flask was equipped with a mechanical stirrer, dropping funnel, thermometer and a N_2-inlet. The dropping funnel was equipped with a $CaCl_2$-tube. The solution was cooled down to 10°C in an ice bath and a solution of 2.7 ml of H_2O in dioxane, see table 1) was added slowly so that the temperature was kept between 10-15°C. The reaction mixture was stirred for additional 45 min. at room temperature, until the initially colorless solution turned pale yellow. This catalyst solution was transferred to the autoclave, treated with 40 g of CO_2 and heated at 125°C for the time indicated in table 1. The mixture was then cooled down to room temperature and 560 g (12.7 mole) CO_2 was added, followed by slow addition of 132 g (3 mole) of ethylene oxide over a time period of 1 hour. The reaction was allowed to proceed for the time indicated in table 1. After this time, the pressure was released slowly during several hours. The product, a sticky slurry, was diluted with dioxane and precipitated by pouring the dioxane solution into 0.25 M of aqueous HCl. The precipitate was dissolved in a proper amount of CH_2Cl_2 (2-4 liter), washed with aqueous 0.5 M HCl (2x) and with H_2O (1x). The solution was dried over anhydrous Na_2SO_4 and evaporated to a final volume of 0.5 to 1.5 liter, depending on the viscosity of the solution. The product was precipitated by pouring the CH_2Cl_2 solution into a 4 fold volume of methanol. The white precipitate was filtered off and dried overnight at 0.5 mbar/50°C. The crude product was reprecipitated from acetone for further purification, see table 2. All products provided identical ^{1}H-NMR spectra except the relative intensities of the signals at 3.65, 3.73, 4.29 and 4.37 ppm due to the differences in ethylene carbonate unit content.

TABLE 1: Experiments for the preparation of poly(ethylene carbonate)s

Example	Ethylene oxide [mol]		CO_2 [mmol]	$Zn(C_2H_5)_2$ [ml]	Dioxane [°C]	Temp. [h]	Time
1	3	13.6	158	300	50	64	
2	3	9.1	158	500	20	64	
3	3	13.6	158	300	20		240
4	3	13.6	158	300	20	40	
5	3	13.6	158	300	20	22	
6	3	13.6	238	300	50	64	

All experiments were run in a 1.0 litre autoclave NB2. Mol ratio H_2O: $Zn(C_2H_5)_2$ = 0.95 for all experiments. The catalyst was pre-treated with 40 g of CO_2 at 125°C for 1 hour except in Example 1 (10 hours).

TABLE 2: Selected physical properties of the synthesized poly(ethylene carbonate)s

Example	Mw [kDa]	Mn [kDa]	Mw/Mn	Tg [°C]	η_{inh} [dl/g] in $CHCl_3$ a)	Ethylene Carbonate Content [%]
1	141.9	32.2	4.40	19.3	0.6087	
2	627.3	133.5	4.70	23.5	1.4691	
3	477.0	83.6	5.71	18.7	1.2791	
4	758.0	97.5	7.77	20.6	1.7590	
5	721.6	80.7	8.95	22.9	2.44 b)	90

6	310.9	103.1	3.02	20.1	88

a) at 20°C and a concentration of 10 mg/ml if nothing else is indicated

b) at a concentration of 1 mg/ml

EXAMPLE 7-11: General procedure for the synthesis of poly(ethylene carbonate)s with a catalyst prepared from diethylzinc and a diol

1. Preparation of the catalyst

200 ml of dry dioxane were placed in a dry, 4-necked 750 ml flask under a nitrogen atmosphere. 19.50 g (158 mmol) diethylzinc were added by the mean of a glass syringe. The flask was equipped with a mechanical stirrer, dropping funnel, thermometer and an argon inlet. The dropping funnel was charged with 100 ml of dry dioxane and equipped with a calcium chloride tube. The apparatus was then set under an argon stream. 9.00 g (145 mmol, 0.92 molequiv.) of fresh distilled, dry ethylene glycol, (kept on molecular sieves) were added to the dioxane in the dropping funnel under an argon stream. The mechanically stirred flask was cooled down to 10°C in an ice bath while under argon. The solution of ethylene glycol in dioxane was added dropwise to the stirred solution of diethyl zinc in dioxane over a time period of 30 minutes, during which time the temperature was kept between 10-14°C. An evolution of ethane gas and precipitation was observed simultaneously on addition of the ethylene glycol solution. After the addition was completed, the cooling bath was removed and the mixture was stirred for additional 60 minutes, while allowing to warm up to room temperature. The heterogeneous mixture was then transferred to an autoclave (1 litre autoclave NB2) while under argon. The autoclave was charged with ca. 40 g (0.9 mol) of carbon dioxide and heated at 125°C for 1 hour under stirring to pre-treat the catalyst with carbon dioxide.

2. Polymerization

The autoclave with the pre-treated catalyst was cooled down to room temperature and was charged with additional 560 g (12.7 mol) of carbon dioxide. Then, 132 g (3 mol) of ethylene oxide (99.8%) were added to the stirred mixture in the autoclave by slow injection

during 1 hour. After the addition was completed, the autoclave was heated to the temperature indicated in table 3 and the mixture was stirred for the given time at this temperature.

3. Work-up

The autoclave was cooled down to room temperature and the pressure was released slowly to atomospheric pressure. The product, a white, sticky slurry, was taken up in a total of 7 liter of dichloromethane, 1035 ml of a 0.4 M HCl solution were added and the mixture was stirred for 3 hours at room temperature. The phases were separated and the organic layer was washed twice with 3 liters of 0.5 M HCl and twice with 4.5 liters of water. The dichloromethane solution was then dried on 120 g of sodium sulfate and concentrated to a final volume of ca. 2 liters. The product was precipitated by slow addition of this solution into 6 liters of methanol. The precipiate was dried 16 hours in vacuo at 40°C to give the crude polymer, which was purified further as follows:

The crude product was dissolved in dichloromethane and the solution was poured into a 5 fold volume of aceton during 15 minutes to precipitate the product. The precipitate was dried 16 hours in vacuo at 40°C to give the corresponding poly(ethylene carbonate). The physical properties of the products are set forth in Table 4. All products showed strong IR-absorptions at 1750 and 1225 cm-1. The 1H-NMR-signal of the ethylene carbonate units appeared at 4.37 ppm.

TABLE 3: Synthesis of poly(ethylene carbonate)s with a catalyst prepared from diethylzinc and a diol

Example	Ethylene Oxide [mol]	CO_2[mol]	Solvent[a)] [ml]	$(C_2H_5)_2Zn$ [mmol]	Diol[b)] [mmol]	Reaction tempera-ture [°C]	Reaction time [hrs]
7	3,0	13,6	300	158	145	20	96
8	3,0	13,6	300	158	145	50	96
9	3,0	13,6	300	158	145	60	96
10	3,0	13,6	300[c)]	158	145	50	144
11	3,0	13,6	300	158	145[d)]	50	96

a) Dioxan, if nothing else indicated

b) Ethylene glycol, if nothing else indicated

c) Tetrahydrofuran as solvent instead of dioxan

d) 1,4-Butandiol instead of ethylene glycol

TABLE 4: Selected physical properties of poly(ethylene carbonate)s synthesized using a catalyst prepared from diethylzinc and a diol

Example	Mw [kDa]	Mn [kDa]	Mw/Mn	Tg [°C]	$\eta_{inh.}$[dl/g] in $CHCl_3$[a)]	ethylene carbonate content [%]
7	-	-	-	16.7	2.88[b)]	98
8	328.0	149.0	2.20	16.4	0.97	95
9	207.0	103.0	2,00	21.2	0.65	92
10	231.0	83.8	2.76	32.6	0.72	96

11	110.0	53.4	2.06	31.1	0.49	90

a) at 20°C and a concentration of 10 mg/ml, if nothing else is indicated

b) at a concentration of 1 mg/ml

EXAMPLE 12: Experimental procedure for the synthesis of poly(ethylene carbonate) with a catalyst prepared from diethylzinc and phloroglucin

1. Preparation of the Catalyst

200 ml of dry dioxane were placed into a dry, 4-necked 750 ml flask under a nitrogen atmosphere. 19.60 g (158.7 mmol) of diethylzinc were added by the mean of a glass syrige. The flask was equipped with a mechanical stirrer, dropping funnel, thermometer and an argon inlet. The dropping funnel was charged with 100 ml of dry dioxane and equipped with a calcium chloride tube. The apparatus was set under an argon stream. 13.34 g (105.8 mmol, 0.92 molequiv.) of dry phloroglucin in the dropping funnel were added to the dioxane under an argon stream. The mechanically stirred flask was cooled down to 10°C in an ice bath while under argon. The solution of phloroglucin in dioxane was added dropwise to the stirred solution of diethylzinc in dioxane over a time period of 30 minutes, during which time the temperature was kept between 10-14°C. An evolution of ethane gas and precipitation was observed simultaneously on addition of phloroglucin solution. After the addition was completed, the cooling bath was removed and the mixture was stirred for additional 30 minutes, while allowing to warm up to room temperature. The heterogeneous mixture was then transferred to an autoclave (1 liter autoclave BN2) while under argon. The autoclave was charged with ca. 40 g (0.9 mol) carbon dioxide and heated at 125°C for 1 hour under stirring to pretreat the catalyst with carbon dioxide.

2. Polymerization

The autoclave with the pre-treated catalyst was cooled down to room temperature and was charged with additional 560 g (12.7 mol) of carbon dioxide. Then, 132 g (3 mol) of ethlyene oxide (99.8%) were added to the stirred mixture in the autoclave by slow injection

during 1 hour. After the addition of ethylene oxide was completed, the autoclave was stirred at 21°C for 260 hours.

3. Work-up

The pressure of the autoclave was released slowly to atmospheric pressure. The product was taken up in a total of 4 liter of dichloromethane, 1035 ml of a 0.4 M HCl solution were added and the mixture was stirred for 3 hours at room temperature. The phases were separated and the organic layer was washed twice with 1.5 liters of 0.5 M CHl and tiwce with 2 liters of water. The dichloromethane solution was then dried on 120 g of sodium sulfate and concentrated to a final volume of ca. 1 liter. The product was precipitated by slow addition of this solution into 3 liters of methanol. The precipitate was dried 16 hours in vacuo at 40°C to give the crude polymer, which was purified further as following:
The crude product was dissolved in dichloromethane and the solution was added into a 5 fold volume of aceton during 15 minutes to precipiate the product. The precipitate was dissolved again in dichloromethane, reprecipitated from methanol and dried 16 hours in vacuo at 40°C to give the corresponding poly(ethylene carbonate).

Physical properties of the product:

Mw = 258000 Da, Mn = 35600 Da, Tg = 15.4°C.

IR: Strong absorbtions at 1751 and 1225 cm-1.

According to 1H-NMR, the product had an ethylene carbonate content of ca. 96%.

EXAMPLE 13: Experimental procedure for the synthesis of poly(ethylene carbonate) with a catalyst prepared from diethylzinc and acetone

132 g (3 Mol) of ethylene oxide were co-polymerized with 600 g (13.6 Mol) CO_2 at 50°C during 96 hrs using a catalyst prepared from 8.43 g (145.16 mmol) of acetone and 19.62 g (159 mmol) of diethylzinc.
The preparation of the catalyst as well as the polymerization were performed similar to the procedure described for examples 7-11, except that acetone was used instead of a diol to prepare the catalyst.
The poly(ethylene carbonate) thus obtained had a ethylene carbonate content of 93% and the following properties:
Mw = 233 kDa, Mn = 109 kDa, Mw/Mn = 2.14, Tg = 22.4°C.

EXAMPLE 14: Synthesis of the endgroup - stearoylated poly(ethylene carbonate)

1 g of poly(ethylene carbonate) having Mw = 153000 Da, Mn = 68900 Da, Tg = 29.1 °C) was dissolved in 30 ml of dry dichloromethane. The solution was treated subsequently with 0.98 g (12.38 mmol) of pyridine and 10 g (33.0 mmol) of stearoyl chloride. The reaction mixture ws stirred at room temperature for 48 hours, than diluted with 50 ml of dichloromethane and washed successively with 2 x 150 ml saturated sodium bicarbonate and water. The organic layer was dried over anhydrous sodium sulfate and the product was precipiated by dropwise addition of the dichloromethane solution into 300 ml of n-hexane. The crude product thus obtained was purified further by dissolving in dichloromethane and precipitation from a 3 fold volume of diethyl ether. Finally, the product was dried in vacuo at 40°C for 16 hours to give the endgroup - stearoylated poly(ethylene carbonate).

Mw = 144000 Da, Mn = 71000 Da, Tg = 25.6°C.

EXAMPLE 15: Synthesis of the endgroup - acetylated poly(ethylene carbonate)

1 g poly(ethylene carbonate) (having Mw = 153000 Da, Mn = 68900 Da, Tg = 29.1°C) was dissolved in 10 ml of dry dichloromethane. 0.98 g (12.38 mmol) of pyridine were added, followed by the addition of 10.08 g (98.7 mmol) of acetic anhydride. The reaction mixture was stirred at room temperature for 120 hours. Then, it was diluted with 50 ml of dichloromethane and was poured slowly onto 200 ml of saturated sodium bicarbonate. The mixture was stirred for 30 minutes and then the layers were separated. The organic layer was washed again with 150 ml of saturated sodium carbonate and finally with water. The dichloromethane solution was dried over anhydrous sodium sulfate and the product was precipitated by dropwise additional of this solution into 300 ml of diethyl ether.
The precipitate was dissolved again in dichloromethane and reprecipitated from diethyl ether. The product was dried for 16 hours at 40°C in vacuo to give the poly(ethlyene carbonate) with the terminal acetate ester group.

Mw = 150000 Da, Mn = 69100 Da, Tg = 26.8°C.

EXAMPLE 16: Purification of poly(ethylene carbonate) by treatment with boiling water

1 g of poly(ethylene carbonate) (of Example 8 having Mw = 328000 Da, Mn = 149000 Da, Tg = 16.4°C) were cut into small pieces and stirred in 50 ml of boiling bidest water for 2 hours. The water was removed and replaced by fresh water, which was heated again to boiling temperature. After additional 3 hours, the polymer pieces were isolated and dried in vacuo at 40°C for 16 hours. The product obtained had the following physical properties: Mw = 340000 Da, Mn = 148000 Da, Tg = 28.3°C. Thus, a dramatic increase of the glass transition temperature was observed which is not attributable to a change in the molecular weight of the polymer.

EXAMPLE 17: Composition (microparticles) with 1% hIL-3 drug loading

1. Preparation of drug containing microparticles

1 g of poly(ethylene carbonate), Mw = 328.000 of Example 8 (PEC) was dissolved in 10 ml of methylene chloride while stirring, followed by the addition of 12.1 mg of human interleukin 3 (hIL-3) dissolved in 0.6 ml of water. The mixture was intensively mixed with the Ultra-Turrax for one minute at 20,000 rpm (= inner W/O-phase). 1 g of Gelatine A was dissolved in 2000 ml of deionized water at 50°C and the solution was cooled down to 20°C (= outer W phase). The W/O-phase and the W-phase were intensively mixed. Thereby the inner W/O-phase was dispersed homogenously in the outer-W-phase to fine droplets. The resulting triple emusion was slowly stirred for 1 hour. Hereby the methylene chloride was evaporated and microparticles were generated from the droplets of the inner phase and hardened.

After sedimentation of the microparticles the supernatant was sucked off and the microparticles were recovered by vacuum filtration or centrifugation and rinsed with water to eliminate gelatine. Finally, microparticles were either freeze-dried by using mannitol as a bulking agent or dried in a vacuum oven (mannitol free formulations) for 72 hours and sieved (0.125 mm mesh size) to obtain the final product.

2. Placebo formulation

1 g of PEC Mw = 328.000 of Example 8 was dissolved in 10 ml of methylene chloride while stirring (inner O-phase). 1 g of Gelatine A was dissolved in 2000 ml of deionized water at 50°C and the solution cooled down to 20°C (=outer W phase). The O- and the W-phase were intensively mixed. Thereby the O-phase was homogenously dispersed to fine droplets in the outer W-phase. The resulting emulsion was slowly stirred for 1 hour and treated further in the manner described above.

EXAMPLES 18-26:

All galenical formulations described hereinafter were prepared using PEC's synthesized according to Example 8 in Table 3 and further purified in a manner similar to that, described in Example 16. All of them had a Mw of 300,000 to 450,000, an ethylene carbonate content of more than 94% and a Tg within the range of 18 to 50°C.

EXAMPLE 18: Composition (microparticles) having a 0.2% hIL-2 loading

2.9 mg of human interleukin 2 (h IL-2) was dissolved in 1.5 ml of water and IL-2 containing microparticles were prepared as described in example 17.
The microparticles were freeze-dried by using mannitol as a bulking agent and sieved (0.125 mm mesh size) to obtain the final product.

EXAMPLE 19: Composition (microparticles) having 0.2% hIL-2 loading (water-free)

The formulation was prepared as described in example 18, however, 2,9 mg of human Interleukin 2 were dispersed directly in the organic phase (PEC dissolved in methylene chloride).

EXAMPLE 20: Composition (implants) having a 0.8% hIL-3 loading

1. Compression moulding

25 mg of microparticles, consisting of 100% (w/w) poly(ethylene carbonate) (placebo), 99% (w/w poly(ethylene carbonate) and 1% (w/w) human interleukin-3 or 79.2% (w/w) poly(ethylene carbonate), 20% (w/w) mannitol and 0.8% (w/w) human interleukin-3, were compression moulded for 3 min at 60-70°C and 160 bar to implants (tablets) of 5 mm diameter. The tablets were stored at 4°C in closed glas vials until use for drug release experiments in vitro and in vivo.

2. Drug release experiments in vitro

Three tablets each of mannitol-free and mannitol-containing human interleukin-3 formulations and placebo formulation were shaken at 37°C in synthetic culture medium containing 2.5% (v/v) N-[2-hydroxyethyl]- piperazine-N'-[2-ethanesulfonic acid] (1 m), 10% (v/v) fetal calf serum, and 2% (v/v) penicillin/streptomycin solution. Samples were drawn from the medium at 0.5, 1, 2, 5 h and 1, 2, 3, 7, 14, 20 days and, subsequently, the medium was renewed. Human interleukin-3 content of the samples was measured by ELISA.

3. Drug release experiment in vivo

Male rats, kept under optimal conditions, were anaesthesized by an inhalation narcotic and in each rat one tablet of the human interleukin-3 formulations and the placebo formulation was implanted in a subcutaneous skin pouch. After 1, 4, 7, 14, 21 days the rats were killed by an overdose of the inhalation narcotic. The remaining tablets were taken out, freed from adhering tissue, and dried. Mass loss of the tablets was determined gravimetrically. Subsequently, the human interleukin-3 content of the remaining tablets was measured by HPLC and ELISA.

Example 21:

Composition (w/o/w microparticles) having a 0.0002% - 2% hIL-2 loading

4 g PEC were dissolved in 80 ml of methylene chloride with magnetic stirring. To this solution an appropriate amount of IL-2 (113.2 mg for 2%, 11.32 mg for 0.2% etc.) dissolved in 6 ml of distilled water or water with some drops of ethanol was added. The mixture was intensively mixed with an Ultra-Turax to disperse the IL-2 solution in the polymer phase (= inner W/O phase). 1 g of gelatin A was dissolved in 200 ml of 1/15 M

phosphate buffer (pH 7.4) at 50° C and the solution cooled down to 20°C (= outer W phase). The W/O- and the W-phase were intensively mixed. Thereby the inner W/O-phase was separated into small droplets which were dispersed homogenously in the outer W-phase. The resulting triple emulsion was slowly stirred for 1 hr. Hereby the methylene chloride was evaporated and the microparticles were hardened from the droplets of the inner phase.

After sedimentation (or centrifugation) of the microparticles the supernatant was sucked off and the microparticles were recovered by vacuum filtration and rinsed with water to eliminate gelatin. Finally, microparticles were dried in a vacuum oven for 24 hr and sieved to obtain the final product.

The encapsulation efficiency, tested with HPLC and bioassay, was between 10 and 100%.

Example 22:

Composition (s/o/w microparticles) having a 0.0002% - 2% of IL-2 loading

The formulations were prepared as desribed in Example 21, except that IL-2 was not dissolved in water. Instead of dissolving IL-2, the drug was dispersed directly into the polymer phase (= O-phase). The encapsulation efficiency, tested with HPLC and bioassay, was between 10 and 100%.

Note: The amount of polymer, methylene chloride, water and drug are varied in a broad range without changing the product quality. Higher drug loadings up to 20% are obtained. In the outer phase the gelatin is replaced by other emulsifiers such as polyvinylalcohol etc., and/or the concentration of the emulsifier/buffer are changed. Separation and drying

procedures described are replaced by other well known pharmaceutical techniques such as filtration, lyophilization and spray drying.

Example 23:

Composition (w/o/w and s/o/w microparticles) having an 1% hGM-CSF loading

The preparation was carried out according to the process described in Examples 21 and 22. As described there S/O/W and W/O/W-preparations are prepared. However, the encapsulation efficiency of W/O/W formlulations was 60%, whereas S/O/W formulations showed lower encapsulation efficiencies.

Examples 24:

Composition (w/o/w and s/o/w microparticles) having an 1 to 10% Octreotide-pamoate (SMS-PA) loading

The preparation was carried out according to the method described in Examples 19 and 20. However, SMS-PA is not water soluble. Thus, the drug was dispersed, not dissolved, in water, for W/O/W formulations. The encapsulation efficiency was determined by HPLC and was between 20 and 100%.

Example 25:

Composition (w/o/w and s/o/w microparticles) having an 1 to 10% Octreotide-acetate loading

The preparation was carried out according to the method described in Examples 21 and 22. The encapsulation efficiency was determined by HPLC and was between 2 and 40%, which is clearly lower than for the lipophilic SMS-PA.
Higher values were obtained in S/O/W formulations after using lyophilized active compound material (smaller drug particle).

Example 26: Octreotide pamoate (SMS-PA) release from microparticles in rabbits and implants in rabbits and rats

Subcutaneous implantation of poly(ethylene carbonate) disks or injection of poly(ethylene carbonate) microparticles (drug loading 1.95%) in an amount of about 2 mg of drug substance/kg body weight were performed in male rabbits (chinchilla bastard, body weight about 3 kg) and subcutaneous implantation of disks in male rats (Wistar, body weight about 375 g). Per rat and rabbit amounts of about 40 resp. 300 mg of drug containing polymer in the form of microparticles resp. pressed to an implant or as suspension were administered.

The implants disks for rats and rabbits had a diameter of 0.5 and 1 cm resp. and were produced as described in example 20.

To determine the drug release, blood samples were collected for 14 and 21 days in rats and rabbits resp. and drug residues were measured in implants by radioimmunoassay and HPLC.

A linear correlation of mass loss of poly(ethylene carbonate) and release of SMS-PA could be found (Fig. 13) as shown for high molecular mass hIL-3 (Fig. 11). A maximum of 75% of implanted material was degraded in 3 weeks after administration in rabbits, a maximum of 95% of implanted material was degraded in 2 weeks after administration in rats.

An inflammation reaction (including invasion of polymorphonuclear leucocytes and other cells) is a prerequisite for biodegradation of poly(ethylene carbonate). The course of an inflammation reaction can be expected to be species-specific giving rise to species-specific plasma level profiles of a drug. This was found for SMS-PA (Fig. 12). In rats, biodegradation of poly(ethylene carbonate) is much faster than in rabbits. In rabbits, plasma levels of SMS-PA increase slowly to reach the phase of constant release at about day 9 lasting until at least day 21.

Example 27:

Composition (w/o/w microparticles) having a 0.0002% - 2% rhIL-6 loading

4 g PEC are dissolved in 80 ml of methylene chloride with magnetic stirring. To this solution an appropriate amount of rhIL-6 (113.2 mg for 2%, 11.32 mg for 0.2% etc.) dissolved in 6 ml of distilled water or water with some drops of ethanol is added. The mixture is intensively mixed with an Ultra-Turax to disperse the IL-6 solution in the polymer phase (= inner W/O phase). 1 g of gelatin A is dissolved in 200 ml of 1/15 M phosphate buffer (pH 7.4) at 50° C and the solution cooled down to 20°C (= outer W phase). The W/O- and the W-phase are intensively mixed. Thereby the inner W/O-phase is separated into small droplets which were dispersed homogenously in the outer W-phase. The resulting triple emulsion is slowly stirred for 1 hr. , the methylene chloride is evaporated, and the microparticles are hardened from the droplets of the inner phase.

After sedimentation (or centrifugation) of the microparticles the supernatant is sucked off and the microparticles are recovered by vacuum filtration and rinsed with water to eliminate gelatin. Finally, microparticles are dried in a vacuum oven for 24 hr and sieved to obtain the final product.

The encapsulation efficiency, tested with HPLC and bioassay, is between 10 and 100%.

Example 28:

Composition (s/o/w microparticles) having a 0.0002% - 2% of rhIL-6 loading

The formulations are prepared as described in Example 27, except that IL-6 is not dissolved in water. Instead of dissolving IL-6, the drug is dispersed directly into the polymer phase (= O-phase). The encapsulation efficiency, tested with HPLC and bioassay, is between 10 and 100%.

Note: The amount of polymer, methylene chloride, water and drug are varied in a broad range without changing the product quality. Higher drug loadings up to 20% are obtained. In the outer phase the gelatin is replaced by other emulsifiers such as polyvinylalcohol etc., and/or the concentration of the emulsifier/buffer are changed. Separation and drying procedures described are replaced by other well known pharmaceutical techniques such as filtration, lyophilization and spray drying.

Examples 29-31: Use of IL-6 in treating conditions mediated by TNFα/and or IL-1

Example 29:
Animal model for multiple sclerosis: Chronic relapsing experimentally induced allergic encephalomyelitis model in the Lewis rat (CR-EAE).

Experimentally induced allergic encephalomyelitis (EAE) in the rat is a well studied experimental model for multiple sclerosis in humans. [Paterson, ADV. IMMUNOL. 5 (1966) 131-208; Levine et al., AM. J. PATH. 47 (1965) 61; McFarlin et al, J. IMMUNOL. 113

(1974) 712; Borel, TRANSPLANT & CLIN. IMMUNOL. 13 (1981) 3]. Rats are injected with nerve tissue from another species together with an adjuvant, and the resulting allergic response leads to lesions on the rat nerves which mimic the autoimmune lesions produced in multiple sclerosis. The rats become partially or completely paralyzed, and the severity of the disease is measured with and without administration of the test drugs. A number of drugs, such as steroids and immunosuppressants, are active in slowing the onset of the disease but are not capable of preventing relapses once the disease is established.

The chronic relapsing experimentally induced allergic encephalomyelitis model (CR-EAE) [Feürer, et al., J. NEUROIMMUNOL: 10 (1985) 159-166] is therefore considered a particularly demanding model which closely mimics actual difficulties in treating multiple sclerosis patients who have established disease. In this model, the disease is induced by injection of a mixture of guinea pig spinal cord and Freund's complete adjuvant enriched with Mycobacterium tuberculosis. Typically 75 - 80 % of the sensitized rats develop a CR-EAE showing 2 - 3 clinical relapses during the first 40 days. After 60 - 80 days, approximately 50 % of the rats with CR-EAE have a further relapse which is followed by complete recovery in only 35 % of all cases. The remaining 65 % of these animals show a progressive state of the disease. Drug treatment starts on day 16, after recovery from the first disease bout.

Recombinant human interleukin 6 (rh IL-6, Sandoz) dissolved in saline was injected i.p. every 2nd day starting on day 16 using 10 micrograms of IL-6 per rat (ca. 50 µg/kg). Control animals and animals in the IL-6 group had the usual severe disease bout (acute) at days 11-14. On a scale of severity from 0 = no disease to 4 = complete paralysis of the animal, the control group averaged 3.0 and the IL-6 group averaged 3.2. Application of IL-6 every second day from day 16 to day 30 (7 applications total) resulted in an almost complete inhibition of the disease. Only one out of 5 IL-6 treated rats showed a slight second disease bout (severity 0.4). Five out of 5 control animals had a second disease bout with a mean

severity rating of 1.8 after day 16, and a third disease attack on days 22 - 29. No other relapses were observed in the IL-6 treated group.

Example 30:

Animal model for arthritis: Borrelia-induced arthritis in severe combined immunodeficiency (SCID) mice.

Lyme arthritis (or Lyme disease arthritis) represents a unique form of chronic arthritis because the initiating event is known with certainty. The disease is one of the prominent features induced by infection with the tick-born spirochete Borrelia burgdorferi. The characteristics of synovial lesions in patients with Lyme arthritis resemble closely those in the synovium of patients with rheumatoid arthritis. In both patient groups synovial lining cell hyperthrophy, synovial cell hyperplasia, vascular proliferation and infiltration of mononuclear cells in the subsynovial lining areas can be observed. Many plasma cells, high endothelial venules, scattered macrophages and few dendritic cells are found with intense MHC class II antigen presentation. In addition, cytokines, such as IL-1, IL-6 and TNF-alpha have been detected in synovial fluid from patients with various arthritides, suggesting that these cytokines may contribute to the pathogenesis of joint destruction. Recently, a mouse model for Lyme arthritis has been developed in SCID mice which lack functional T and B cells (M. M. Simon, et al. (1991) Immunology Today 12: 11). The infection of the immunodeficient mice with Borrelia burgdorferi leads to a prominent and persistant oligoarthritis. The Borrelia-induced arthritis in SCID mice responds to corticosteroids (prednisolon 30 mg/kg sc) but not to immunosuppressive agents like SIM (cyclosporin A) up to doses of 30 mg/kg s.c. It is considered a good model for cytokine-driven arthritis, including other types of arthritis for which the initiating event is not known with certainty.

Six week old C.B-17 SCID mice (homozygous for the SCID mutation, obtained from Bomholtgard, Denmark, 5-6 animals/group) were inoculated with 100 mio. Borrelia

burgdorferi organisms by s.c. tailbase injection. Immunocompetent C.B-17 mice (same source) were used as control animals. They do not develop any disease upon injection of Borrelia burgdorferi. Recombinant human IL-6 (rhIL-6, Sandoz, stock sol. 5 mg/ml) was diluted with physiological saline and was given 5 times per week for a total of 17 injections at a dose of 10 microgram/mouse i.p. Mice were monitored daily in blinded fashion for clinical signs of arthritis in the tibiotarsal and ulnacarpal joints. Clinical arthritis was scored according to the following parameters:

- no signs
? signs questionable
(+) reddening of joints
+ slight swelling
++ moderate swelling
+++ severe swelling of the tibiotarsal and ulnacarpal joints.

At the peak of clinical arthritis, mice were sacrificed and the joints were fixed in Schaffer's solution, embedded in plastic 9100 and stained with hematoxilin eosin.

Group	clinical signs (number of swollen joints/total) on days					
	13	14	15	16	17	20
Control	0/30	0/30	0/30	0/30	0/30	0/30
SCID, no IL-6	6.5/36	12.5/36	15/36	21/36	30/36	35/36
% w. arthrit.	18 %	35 %	42 %	58 %	83 %	97 %
SCID, IL-6 treated	4/30	3.5/30	11/30	7.5/30	12/30	10.5/30
% w. arthrit.	13 %	12 %	37 %	25 %	40 %	35 %

SCID mice which are not treated with IL-6 develop severe arthritis due to infection with Borrelia burgdorferi starting around day 13 after antigen injection. A low dose of rh IL-6 reduces the severity of arthritis by an average of 60 - 75 % in all afflicted animals.

Example 31: Murine model for septic shock

It was decided to investigate the effects of IL-6 in the mouse endotoxic shock model using d-galactosamine sensitized mice, since this is widely used as a model for human septic shock. Our methods and result are as follows:

Female OF1 mice weighing 18-22 g, were challenged with a 0.2 ml i.p. injection of a PBS solution containing 0.15 mg/kg lipopolysaccharide endotoxin (LPS) and 500 mg/kg d-galactosamine. Mice were divided in groups of 10 mice each and treated as follows:

Experiment 1

Time	11:00	14:00	16:00
Group 1:	PBS	LPS + d-GAL	PBS
Group 2:	IL-6 (50 µg)	LPS + d-GAL	PBS
Group 3:	PBS	LPS + d-GAL + IL-6 (50 µg)	PBS
Group 4:	PBS	LPS + d-GAL	IL-5 (50 µg)

Experiment 2

Time	11:00	14:00	16:00
Group 1:	PBS	LPS + d-GAL	PBS
Group 2:	IL-6 (100 µg)	LPS + d-GAL	PBS
Group 3:	PBS	LPS + d-GAL + IL-6 (100 µg)	PBS

Group 4:	PBS	LPS + d-GAL + IL-6 (20 µg)	PBS
Group 5:	PBS	LPS + d-GAL + IL-6 (5 µg)	PBS
Group 6:	PBS	LPS + d-GAL + IL-6 (0.8 µg)	PBS
Group 7:	PBS	LPS + d-GAL + IL-2 (100 µg)	PBS
Group 8:	PBS	LPS + d-GAL + IL-4 (50 µg)	PBS
Group 9:	PBS	LPS + d-GAL	IL-6 (100 µg)

rhIL-6 (ILS 969, Sandoz), rhIL-2 (Sandoz) and rhIL-4 (Sandoz) were diluted in PBS. All injections (0.2 ml volume) were given intraperitoneally. In group 3 (exp. 1) and group 3 to 8 (exp. 2) IL-6 and IL-2 were diluted into the LPS/d-GAL solution so that mice received a single 0.2 ml injection. Numbers in parenthesis indicate the dose of interleukin given to each mouse. The multiple dosing of PBS was required to control inter-group variability due to stress induced responses due to handling at different times prior or post LPS challenge.

Mouse survival was observed for 48 hours. For statistical calculation, we used the Chi square test. After 24 hours from LPS challenge, 9 out of 10 control mice died. IL-6 treatment 3 hours prior to LPS injection or 2 hours after LPS after LPS injection, reduced the mortality respectively to 60% ($p = 0.12$) and 70% ($p = 0.26$). On the other hand, IL-6 given at the time of LPS challenge reduced the group mortality to 10% ($p< 0.01$). The protective effets were long lasting, since after 48 hours the mortality in group 3 increased slightly , i.e. to 30%, still indicating a highly significant protection respect to the control group ($p<0.01$). The mortality of group 4 passed from 70% to 80%, whereas no changes were observed in group 1 and 2.

Based on these results, we tested the effect of IL-6 at different doses. We gave IL-6 at the time of LPS injection, since according to the first experiment this was the optimal time. We explored as well the effect of IL-2 and IL-4 given at the time of LPS as a way to exclude possible artifacts due to the use of recombinant proteins in the LPS/d-GAL

preparation. We also tested whether IL-6 was effective in protecting mice from endotoxic death a dose of 100 µg/mouse given before or after LPS.

The results of experiment 2 are in line with those of experiment 1. Also in this experiment, treatment with IL-6 protected mice from endotoxic death. When IL-6 was given together with LPS, the resulting protection 24 hours after LPS was dose dependent at the dose of 20 (30% deaths, $p = 0.03$), 4 (50% deaths, $p = 0.16$) and 0.8 (70% deaths, $p = 0.61$) µg/mouse, whereas at the dose of 100 µg/mouse (60% deaths, $p = 33$) mice were protected less efficiently than at a 20 µg/mouse. Pre- or post- treatment with 100 µg IL-6 / mouse resulted in a protection comparable to that observed when the same dose of IL-6 was given together with LPS. Similar mouse survival results were obtained 48 hours after LPS.

IL-4 given at the time of LPS challenge was ineffective in protecting mice from endotoxic death, whereas IL-2 decreased mouse survival.

CLAIMS:

1. A biodegradable polymer, comprisinging ethylene carbonate units of the formula A

$$-(-C(O)-O-CH_2-CH_2-O-)- \qquad A$$

and having an ethylene carbonate content of 70 to 100 Mol%, an intrinsic viscosity of 0.4 to 4.0 dl/g in chloroform and a glass transition temperature of from 15 to 50°C.

2. A polymer according to claim 1, having a molecular weight (Mw) of 100,000 to 2,000,000, determined by gel permeation chromatography, with methylene chloride as the eluant and polystyrene as the reference.

3. A polymer according to claim 1, having an ethylene carbonate content of 90 - 100 Mol %.

4. A polymer according to claim 1, having an inherent viscosity, measured at a concentration of 1 g/dl in chloroform of 0.4 - 3.0 dl/g.

5. A polymer according to claim 1, having a glass transition temperature of 18 to 50°C.

6. A polymer according to claim 1, having ethylene carbonate units and ethylene oxide units.

7. A polymer according to claim 1, which is not significantly degradable during at least 1 month by hydrolytic enzymes under physiological conditions or by water at pH 12 and 37°C.

8. A polymer according to claim 7, which after exposure to boiling bidistilled water during 5 hours, has a glass transition temperature of 18 to 50°C.

9. A polymer according to claim 1, degrading in vivo and in vitro by surface erosion under the influence of the superoxide radical anion $O_2^{\cdot-}$.

10. A polymer according to claim 9, which shows a continuous mass degradation without reduction of molecular weight of the residual mass.

11. A polymer according to claim 10, being biodegradable in a period of time of 5 days to 6 months.

12. A polymer according to any one of claims 1-11, having as a co- unit the ethylene oxide unit of the formula B

$$-(-CH_2-CH_2-O-)- \qquad B$$

13. A polymer according to any one of claims 1-12, having a hydroxyl group as a polymer terminal group.

14. A polymer according to any one of claims 1 to 12 having an ester group as a polymer terminal group.

15. A polymer according to claim 14, with a surface erosion rate adjustable by choice of the terminal ester group.

16. A process for the production of the polymer according to any one of claims 1-12, in which ethylene oxide and CO_2 are polymerized in a molar ratio of from 1:4 to 1:5 under the influence of a catalyst.

17. A process according to claim 16, in which a catalyst is used, prepared from $Zn(C_2H_5)_2$ and water or acetone in a molar ratio of from 0.9:1 to 1:0.9.

18. A process according to claim 16, in which a catalyst is used, prepared from Zn $(C_2H_5)_2$ and a di- or triphenol in a molar ratio of from 2:1 to 1:2.

19. A process according to claim 16, in which a catalyst is used, prepared from Zn $(C_2H_5)_2$ and a diol in a molar ratio of 0.9:1 to 1:0.9.

20. A process according to claim 19 in which a catalyst is used, prepared from $Zn(C_2H_5)_2$ and ethylene glycol.

21. A process according to claim 18, in which a catalyst is used, prepared from Zn $(C_2H_5)_2$ and phloroglucin.

22. A process according to any one of claims 16 to 21 in a solvent or dispersing agent system of an organic solvent and CO_2.

23. A process according to any one of claims 16 - 22, under a pressure of from 20 to 70 bar and a temperature of 10 to 80°C.

24. A process for the production of a polymer having a hydroxyl group as a (co)-polymer terminal group according to claim 13, performed according to any one of claims 17 to 22.

25. A process for the production of a polymer having an ester group as a polymer terminal group according to claim 14, performed according to any one of claims 17 to 22 and optionally completed with an esterification step.

26. A pharmaceutical composition comprising a pharmacologically active compound in a polymer showing non-hydrolytic surface erosion.

27. A pharmaceutical composition of a pharmacologically active compound in a polymer according to claim 26 showing a linear correlation of active compound release and non-hydrolytic polymer mass degradation and active compound protection in the polymer matrix.

28. A pharmaceutical composition in a polymer according to any one of claims 1-15.

29. A pharmaceutical composition according to claim 26 or 27 containing an active protein or peptide.

30. A pharmaceutical composition according to claim 29 containing a cytokine.

31. A pharmaceutical composition according to claim 30 containing an interleukin.

32. A pharmaceutical composition according to claim 26 in the form of microparticles or an implant.

33. A pharmaceutical composition according to any one of claims 26 - 29 containing, in or on the polymer, an additive.

34. A pharmaceutical composition according to claim 33 containing a radical scavenger as the additive.

35. A pharmaceutical composition according to claim 33 containing a polyol as the additive.

36. A pharmaceutical composition according to claim 35 containing a sugar alcohol as the additive.

37. A pharmaceutical composition according to claim 36 containing mannitol as the additive.

38. A pharmaceutical composition according to any one of claims 33-35 containing 1 to 90% of weight of the additive, related to the total weight.

39. A pharmaceutical composition according to claim 26 for parenteral administration of an interleukin or CSF.

40. A pharmaceutical composition claimed in claim 39 wherein the interleukin or CSF is in a polymer having the formula A as defined in claim 1.

41. A method of administering a composition according to claim 39 to a subject which comprises administering it parenterally to a subject in need of such treatment.

42. A pharmaceutical composition according to any one of claims 26-40 comprising IL-6 as an active ingredient.

43. A pharmaceutical composition comprising IL-6 in a polymeric matrix.

44. A pharmaceutical composition according to claim 43 in microparticle or implantable form.

45. A pharmaceutical composition according to claim 44 for treatment of an autoimmune or inflammatory condition.

46. A pharmaceutical composition according to claim 45 when the condition to be treated is multiple sclerosis.

47. A pharmaceutical composition according to claim 45 when the condition to be treated is rheumatoid arthritis.

48. A pharmaceutical composition according to claim 45 when the condition to be treated is Lyme disease.

49. The use of IL-6 in the manufacture of a medicament for downregulation or inhibition of IL-I and/or TNFα.

50. The use of IL-6 according to claim 49 in the manufacture of a medicament for the treatment of a chronic or acute pathogen-induced inflammatory condition or a demyelinating disease.

51. The use according to claim 50 when the disease or condition is septic shock.

52. The use according to claim 50 when the disease or condition is multiple sclerosis.

53. The use of IL-6 according to claim 50 where the disease or condition is Lyme disease.

54. The use according to any one of claims 49-53 when the IL-6 is recombinant human IL-6.

Fig. 1

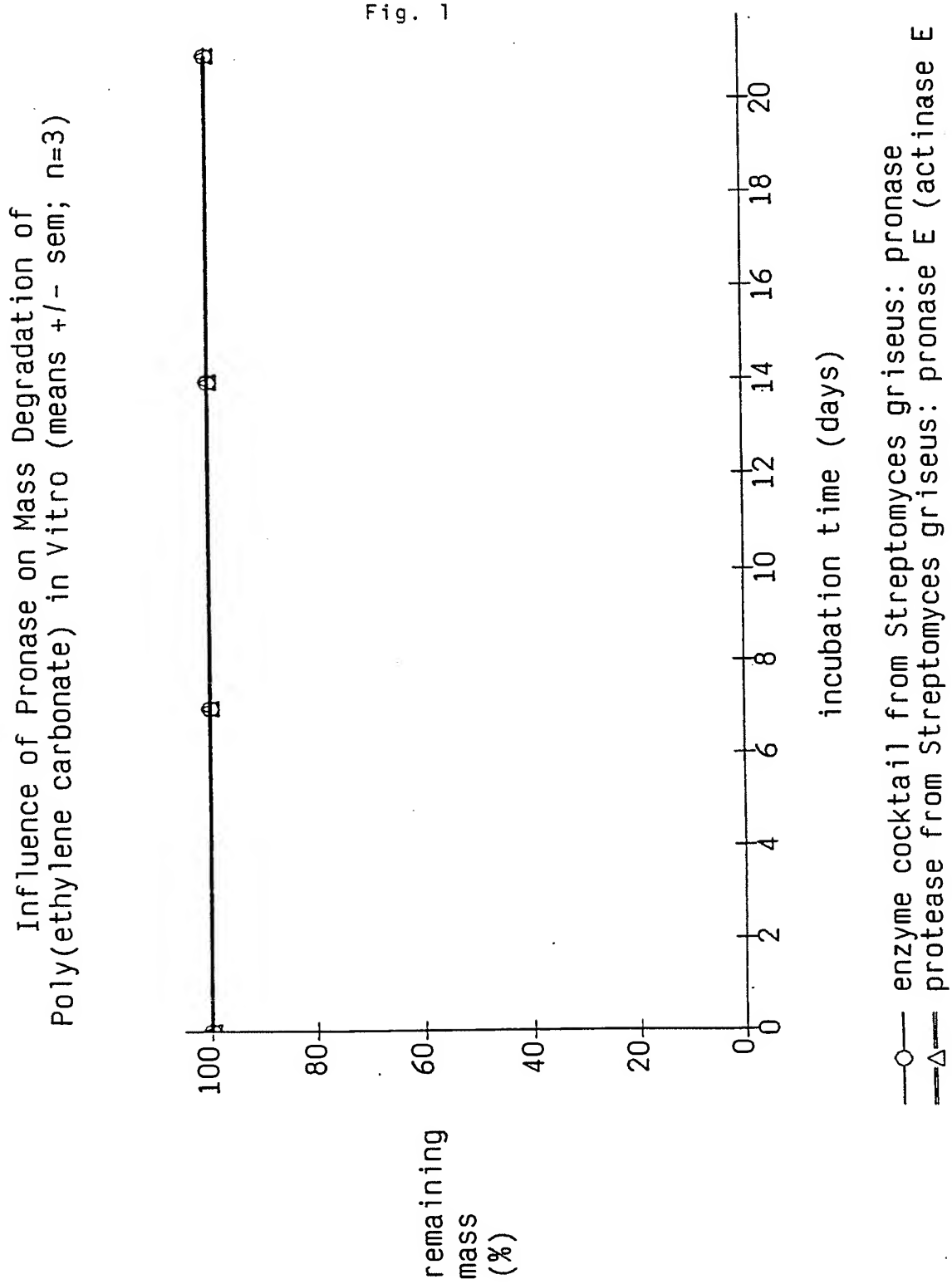

Influence of Pronase on Mass Degradation of
Poly(ethylene carbonate) in Vitro (means +/- sem; n=3)
remaining mass (%)
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
incubation time (days)
enzyme cocktail from Streptomyces griseus: pronase
protease from Streptomyces griseus: pronase E (actinase E

Fig. 2

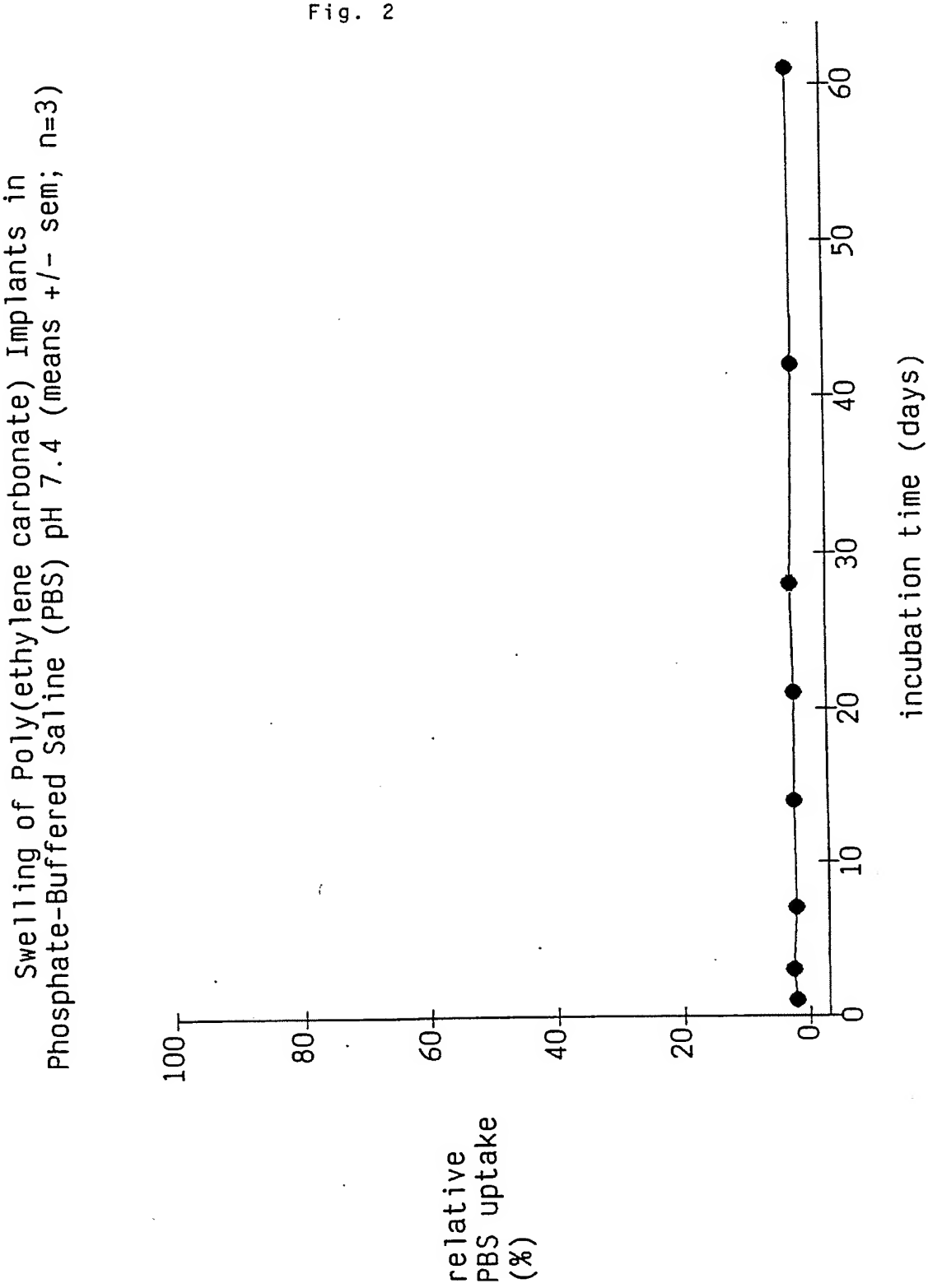

Swelling of Poly(ethylene carbonate) Implants in
Phosphate-Buffered Saline (PBS) pH 7.4 (means +/- sem; n=3)
relative
PBS uptake
(%)
100
80
60
40
20
0
0
10
20
30
40
50
60
incubation time (days)

Fig. 3

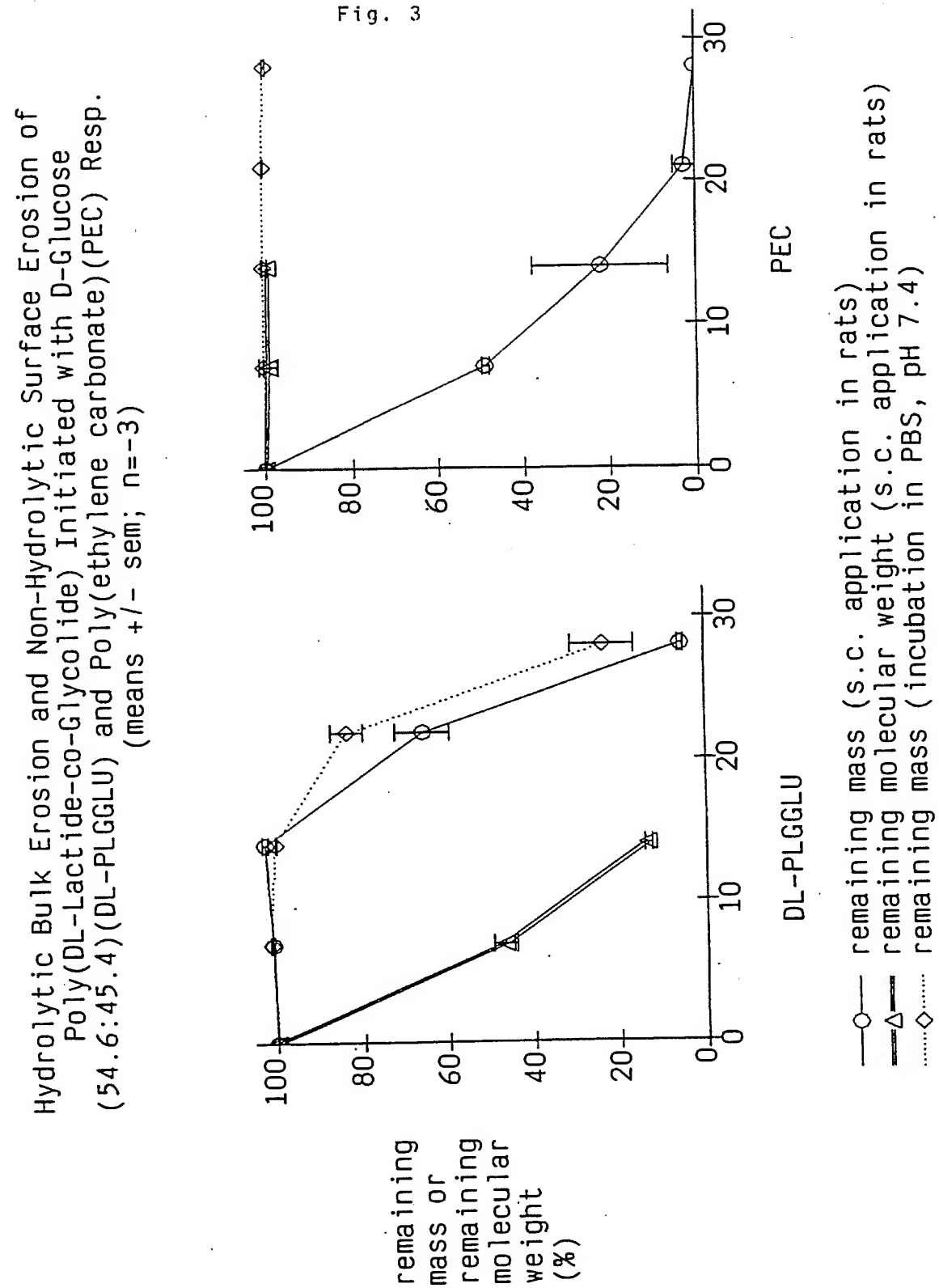

Hydrolytic Bulk Erosion and Non-Hydrolytic Surface Erosion of
Poly(DL-Lactide-co-Glycolide) Initiated with D-Glucose
(54.6:45.4)(DL-PLGGLU) and Poly(ethylene carbonate)(PEC) Resp.
(means +/- sem; n=-3)
remaining mass or remaining molecular weight (%)
0
20
40
60
80
100
0
10
20
30
DL-PLGGLU
PEC
remaining mass (s.c. application in rats)
remaining molecular weight (s.c. application in rats)
remaining mass (incubation in PBS, pH 7.4)

Fig. 4

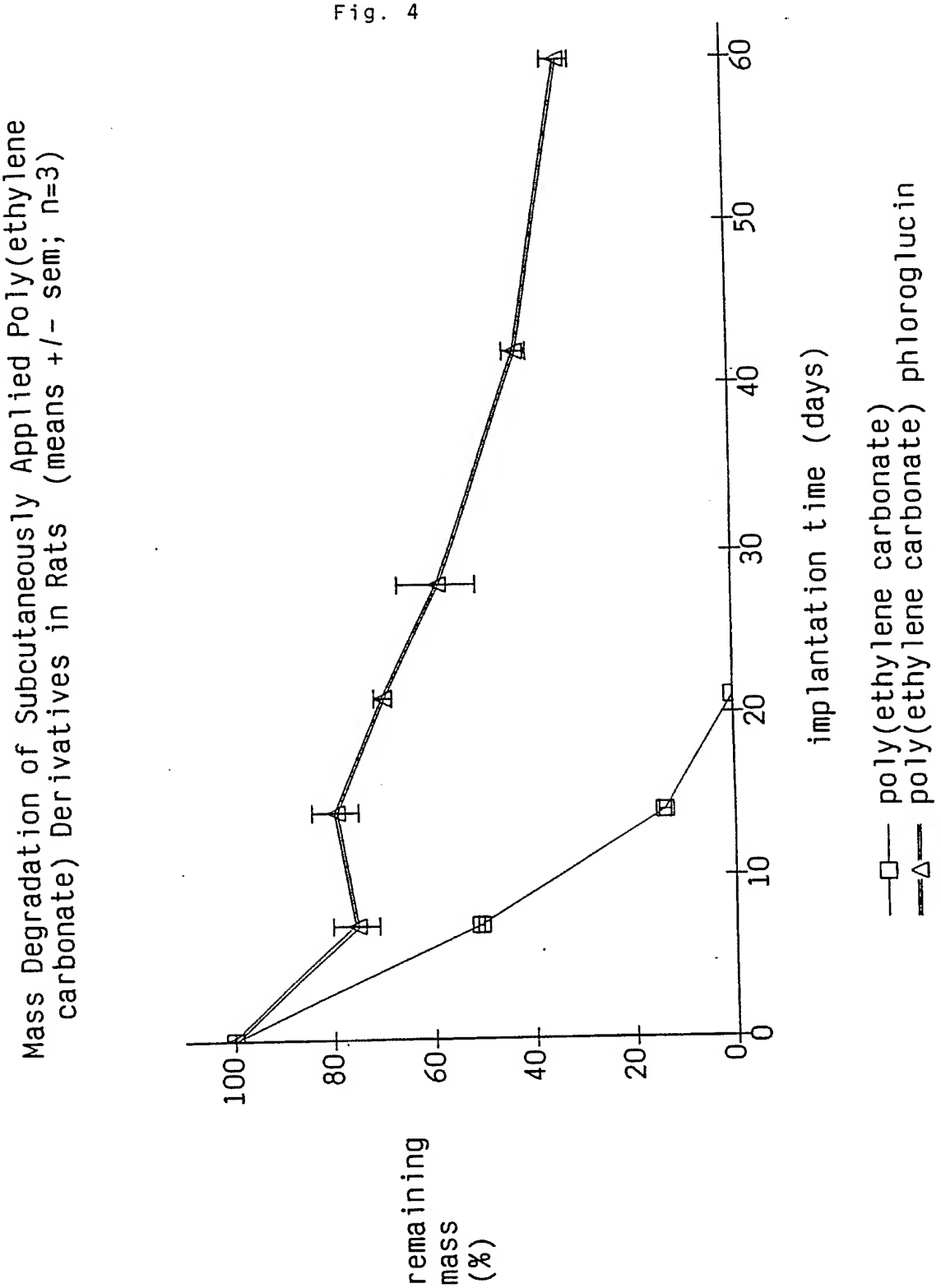

Mass Degradation of Subcutaneously Applied Poly(ethylene carbonate) Derivatives in Rats (means +/- sem; n=3)
remaining mass (%)
100
80
60
40
20
0
0
10
20
30
40
50
60
implantation time (days)
poly(ethylene carbonate)
poly(ethylene carbonate) phloroglucin

Fig. 5

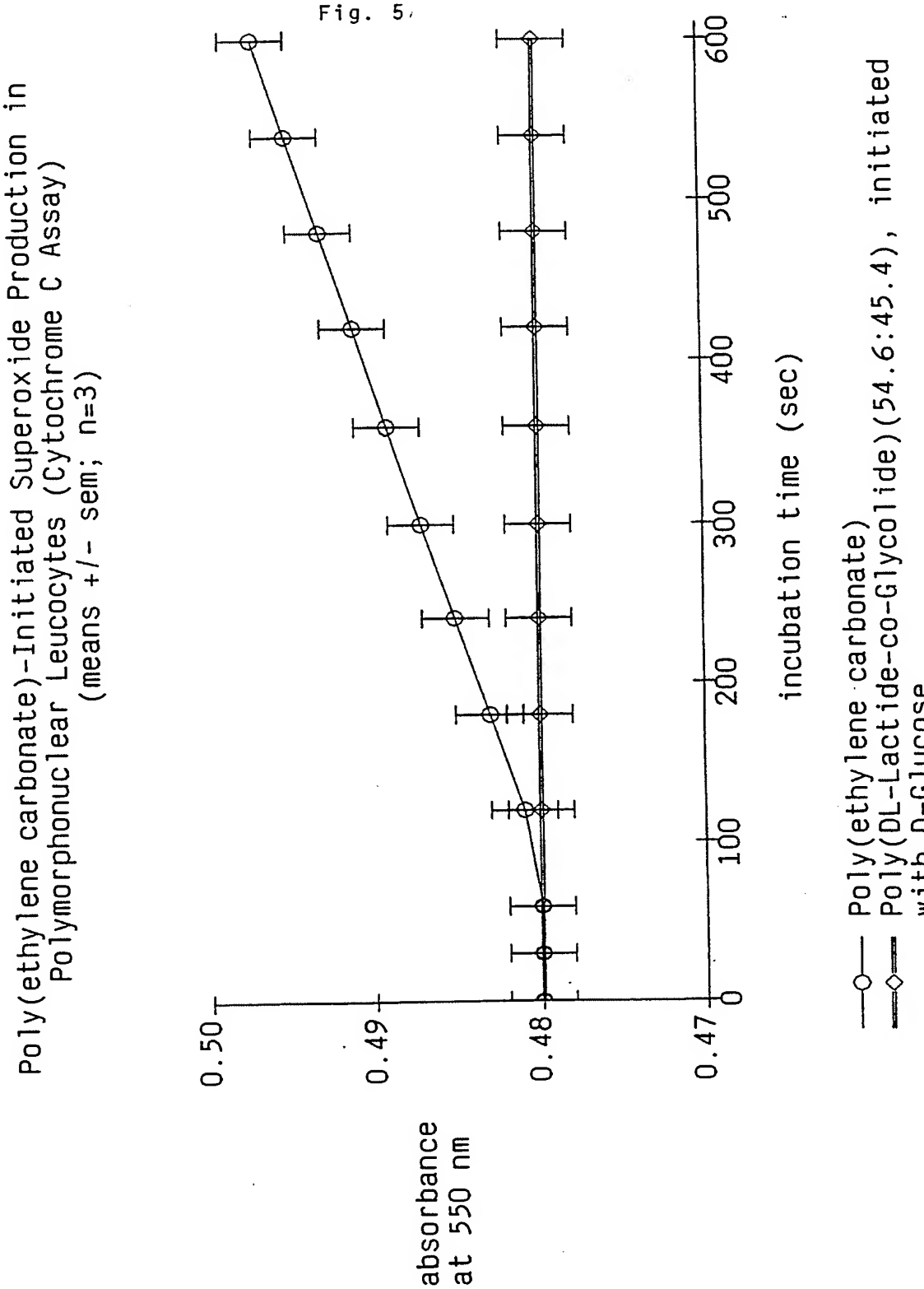

Poly(ethylene carbonate)-Initiated Superoxide Production in Polymorphonuclear Leucocytes (Cytochrome C Assay)
(means +/- sem; n=3)
absorbance at 550 nm
0.50
0.49
0.48
0.47
0
100
200
300
400
500
600
incubation time (sec)
Poly(ethylene carbonate)
Poly(DL-Lactide-co-Glycolide)(54.6:45.4), initiated with D-Glucose

Fig. 6

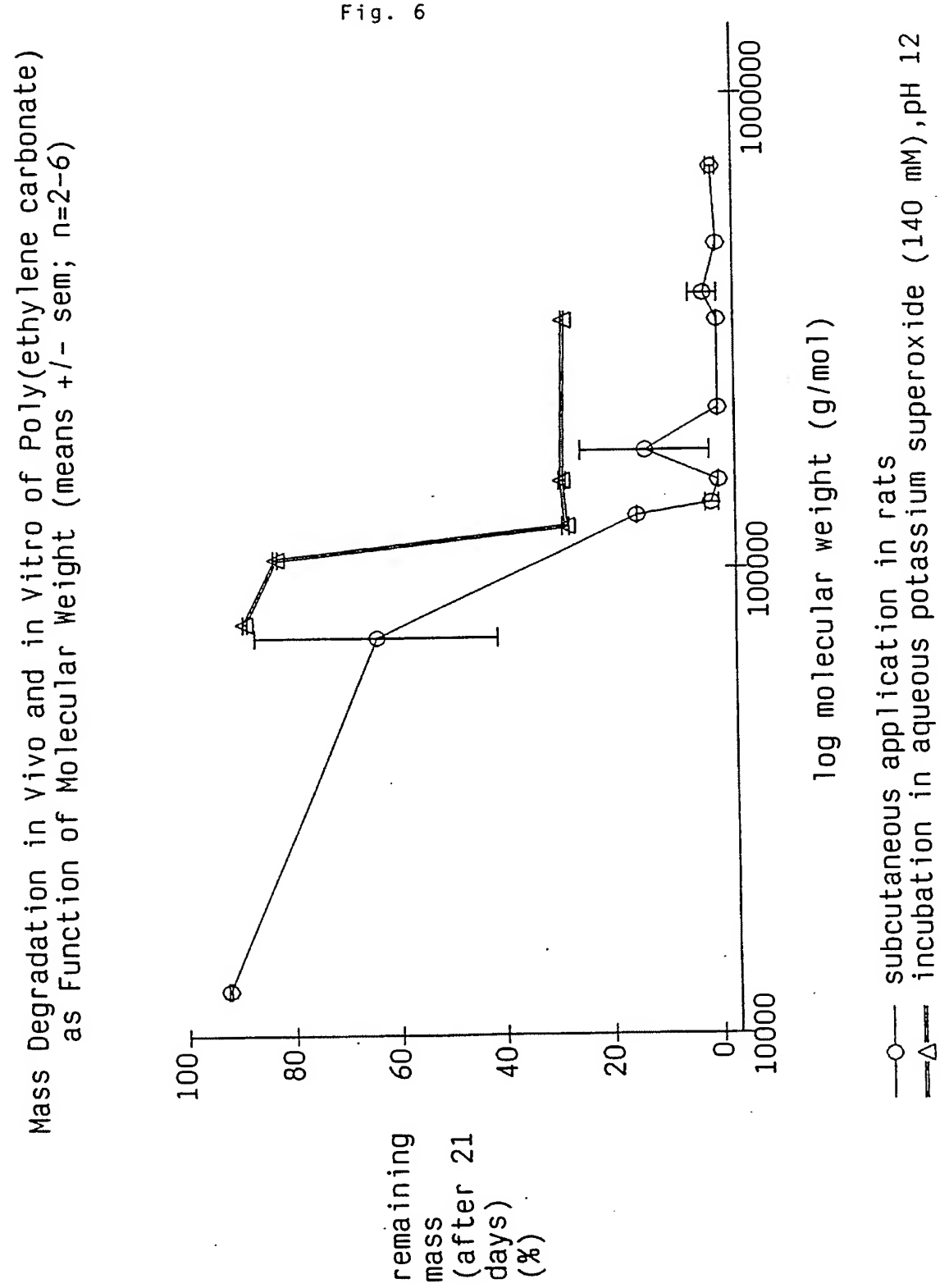

Mass Degradation in Vivo and in Vitro of Poly(ethylene carbonate)
as Function of Molecular Weight (means +/- sem; n=2-6)
remaining mass (after 21 days) (%)
100
80
60
40
20
0
10000
100000
1000000
log molecular weight (g/mol)
subcutaneous application in rats
incubation in aqueous potassium superoxide (140 mM),pH 12

Fig. 7

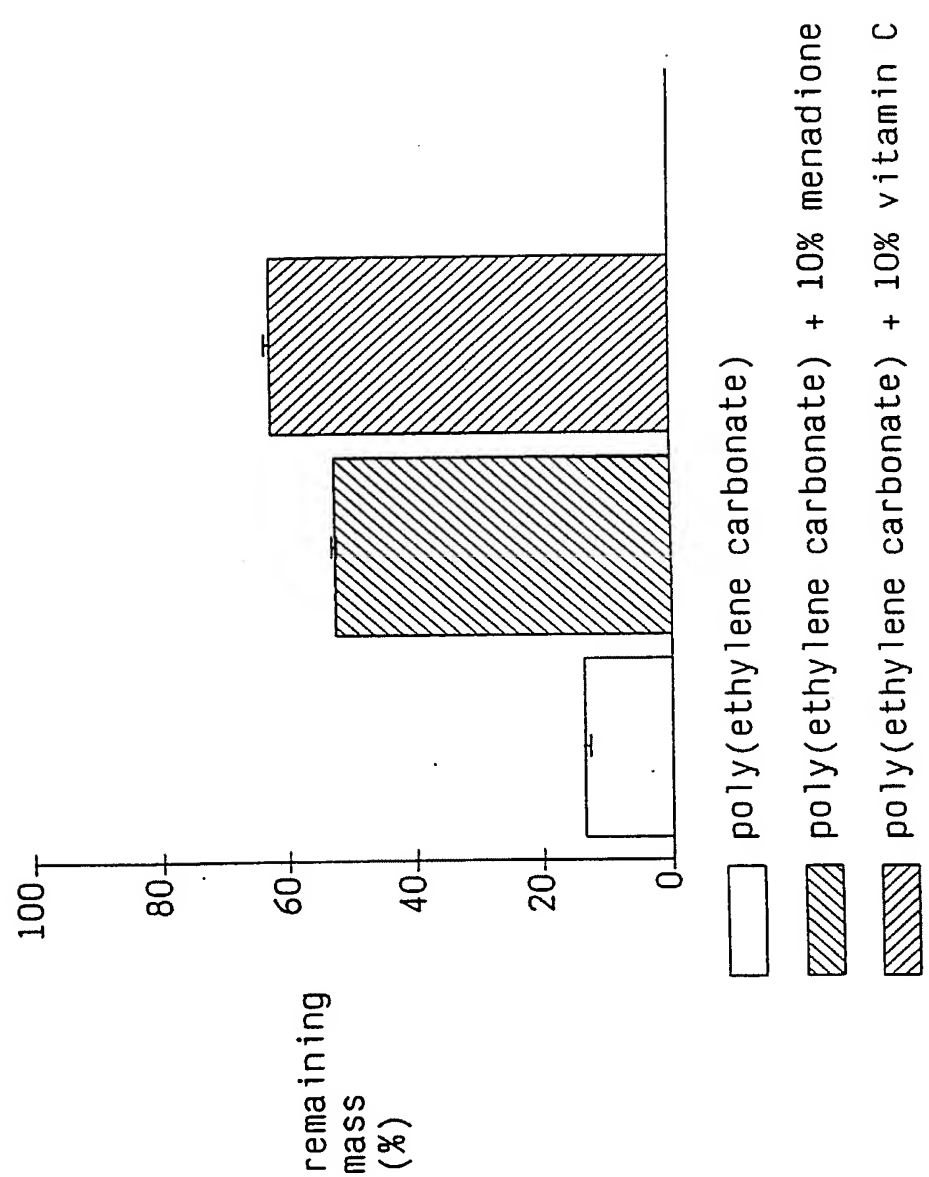

Influence of Superoxide Radical Scavengers on Mass Degradation of Poly(ethylene carbonate) 14 Days after Subcutaneous Application in Rats (means +/- sem; n=3)
remaining mass (%)
100
80
60
40
20
0
poly(ethylene carbonate)
poly(ethylene carbonate) + 10% menadione
poly(ethylene carbonate) + 10% vitamin C

Fig. 8

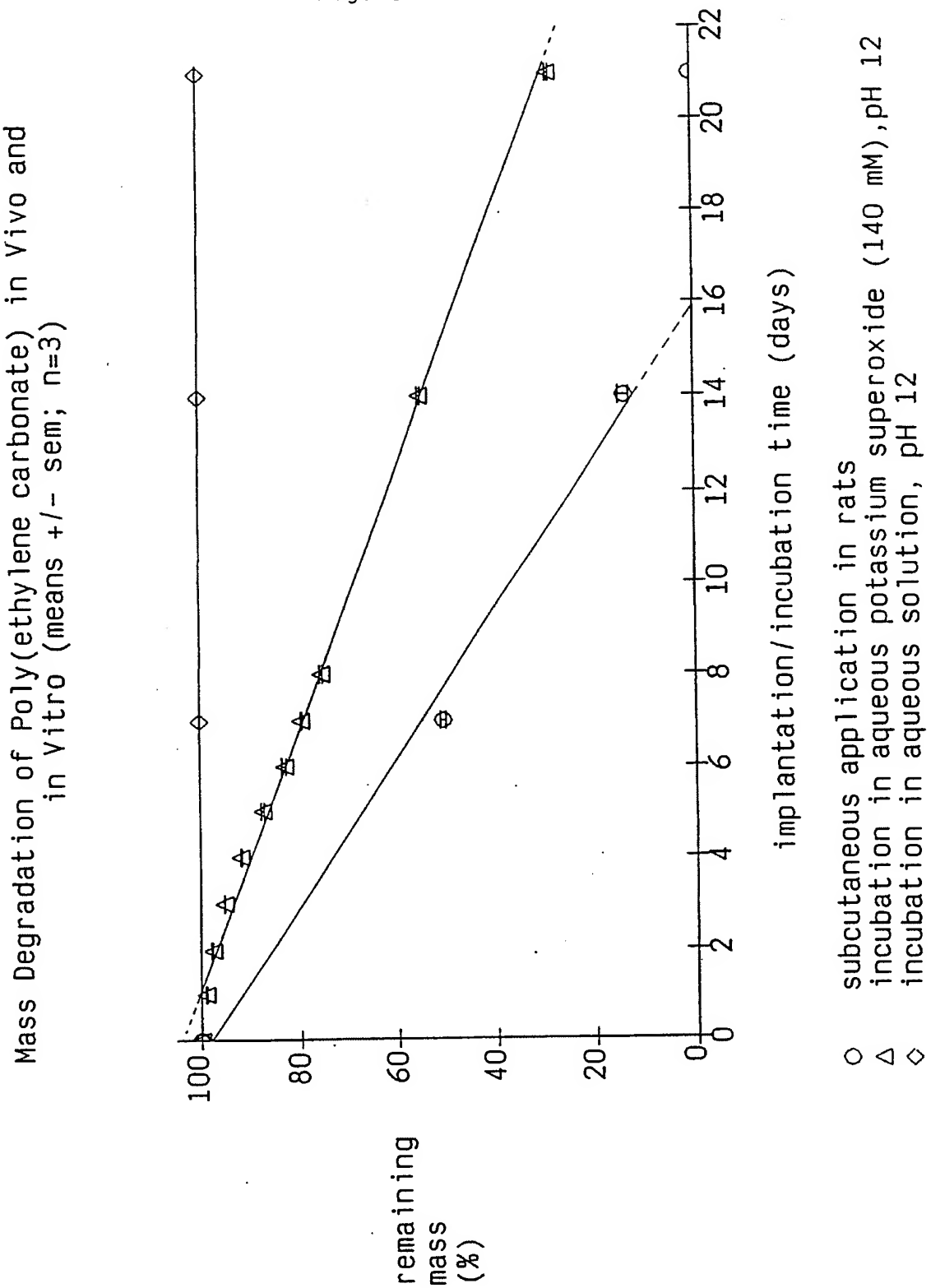

Mass Degradation of Poly(ethylene carbonate) in Vivo and in Vitro (means +/- sem; n=3)
remaining mass (%)
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
22
implantation/incubation time (days)
○ subcutaneous application in rats
△ incubation in aqueous potassium superoxide (140 mM),pH 12
◇ incubation in aqueous solution, pH 12

Fig. 9

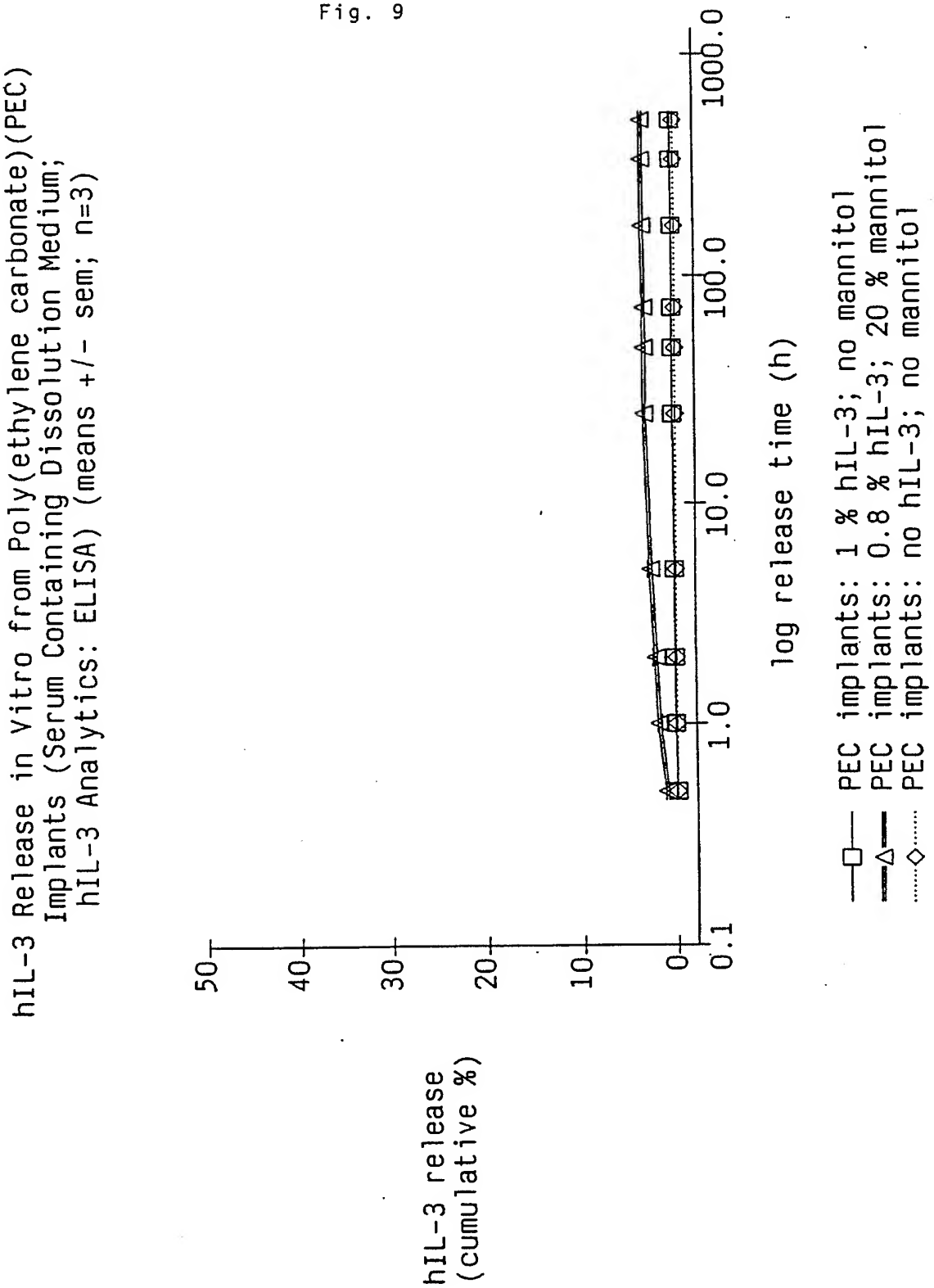

hIL-3 Release in Vitro from Poly(ethylene carbonate)(PEC) Implants (Serum Containing Dissolution Medium; hIL-3 Analytics: ELISA) (means +/- sem; n=3)
hIL-3 release (cumulative %)
50
40
30
20
10
0
0.1
1.0
10.0
100.0
1000.0
log release time (h)
PEC implants: 1 % hIL-3; no mannitol
PEC implants: 0.8 % hIL-3; 20 % mannitol
PEC implants: no hIL-3; no mannitol

Fig.10

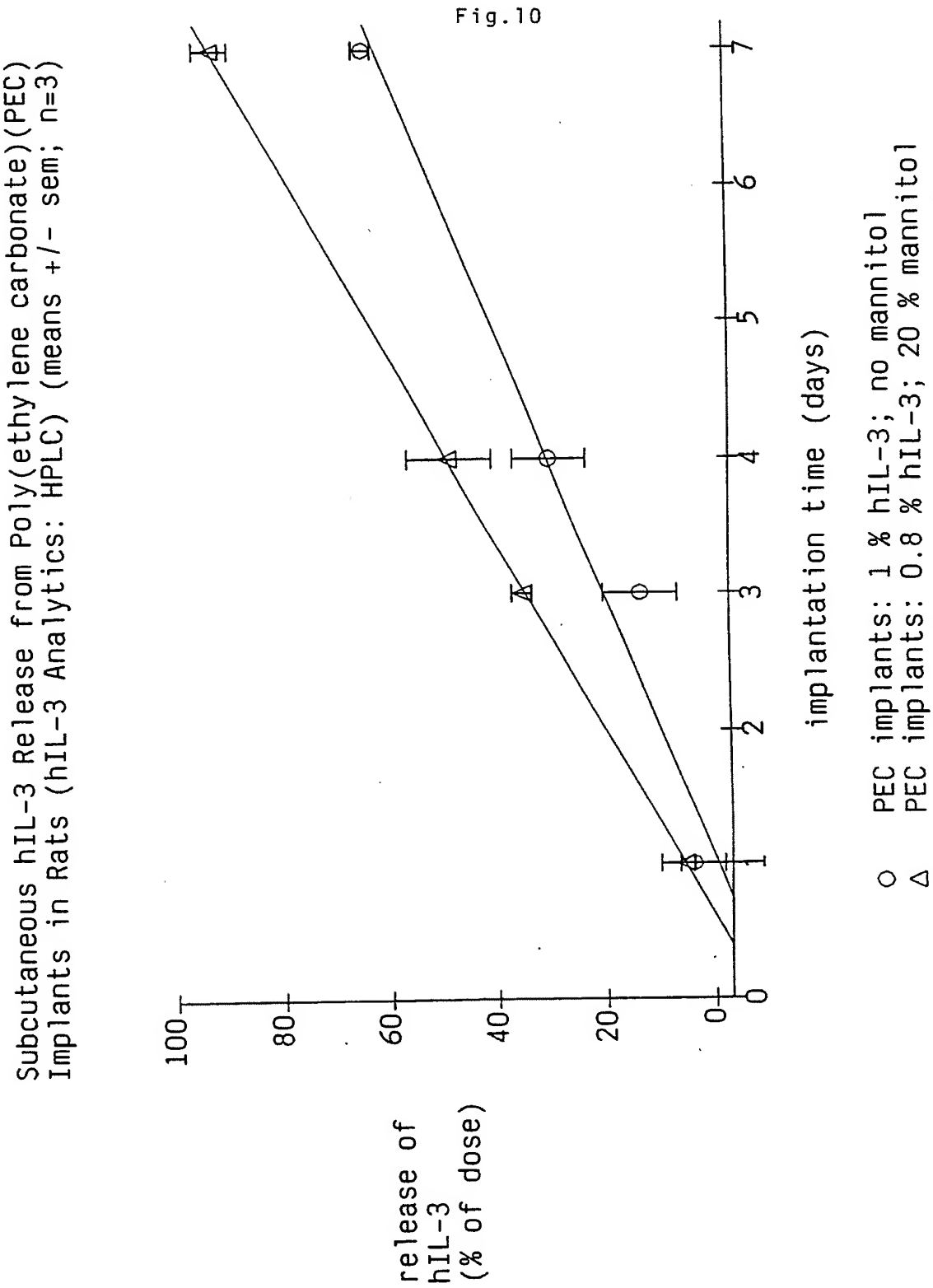

Subcutaneous hIL-3 Release from Poly(ethylene carbonate)(PEC) Implants in Rats (hIL-3 Analytics: HPLC) (means +/- sem; n=3)
release of hIL-3 (% of dose)
100
80
60
40
20
0
0
1
2
3
4
5
6
7
implantation time (days)
○ PEC implants: 1 % hIL-3; no mannitol
△ PEC implants: 0.8 % hIL-3; 20 % mannitol

Fig. 11,

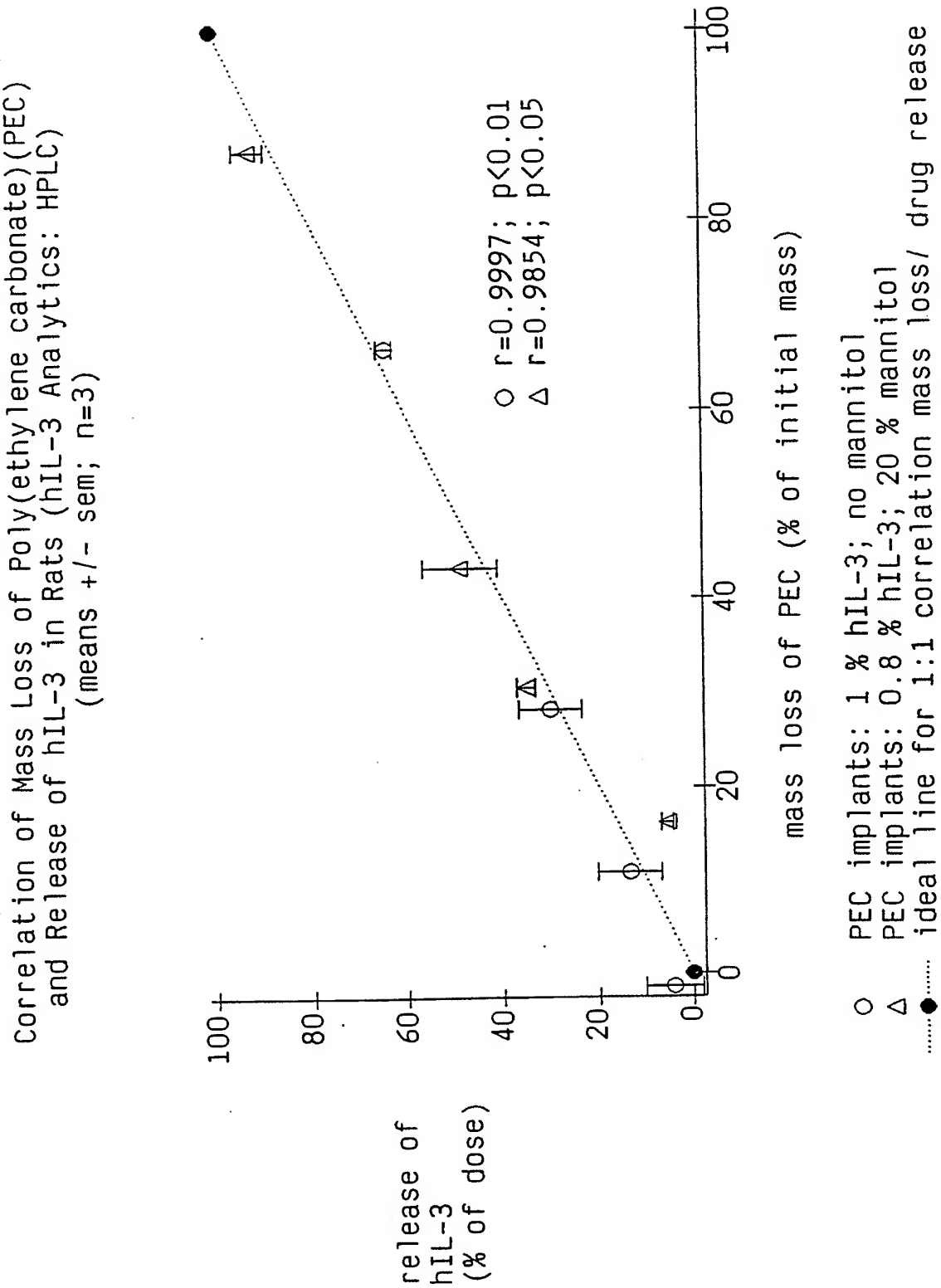

Correlation of Mass Loss of Poly(ethylene carbonate)(PEC)
and Release of hIL-3 in Rats (hIL-3 Analytics: HPLC)
(means +/- sem; n=3)
release of hIL-3 (% of dose)
mass loss of PEC (% of initial mass)
0
20
40
60
80
100
○ r=0.9997; p<0.01
△ r=0.9854; p<0.05
○ PEC implants: 1 % hIL-3; no mannitol
△ PEC implants: 0.8 % hIL-3; 20 % mannitol
ideal line for 1:1 correlation mass loss/ drug release

Fig. 12

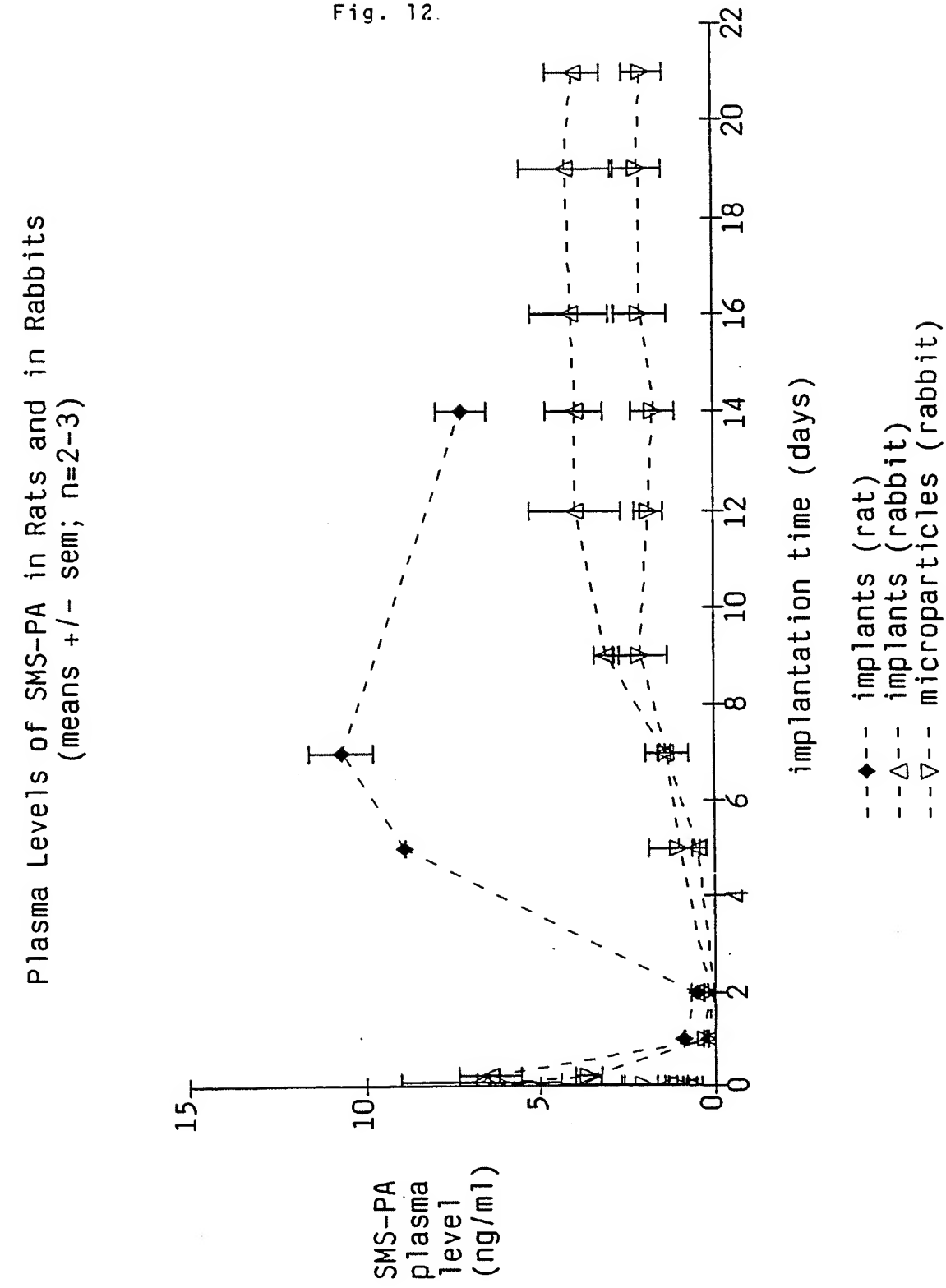
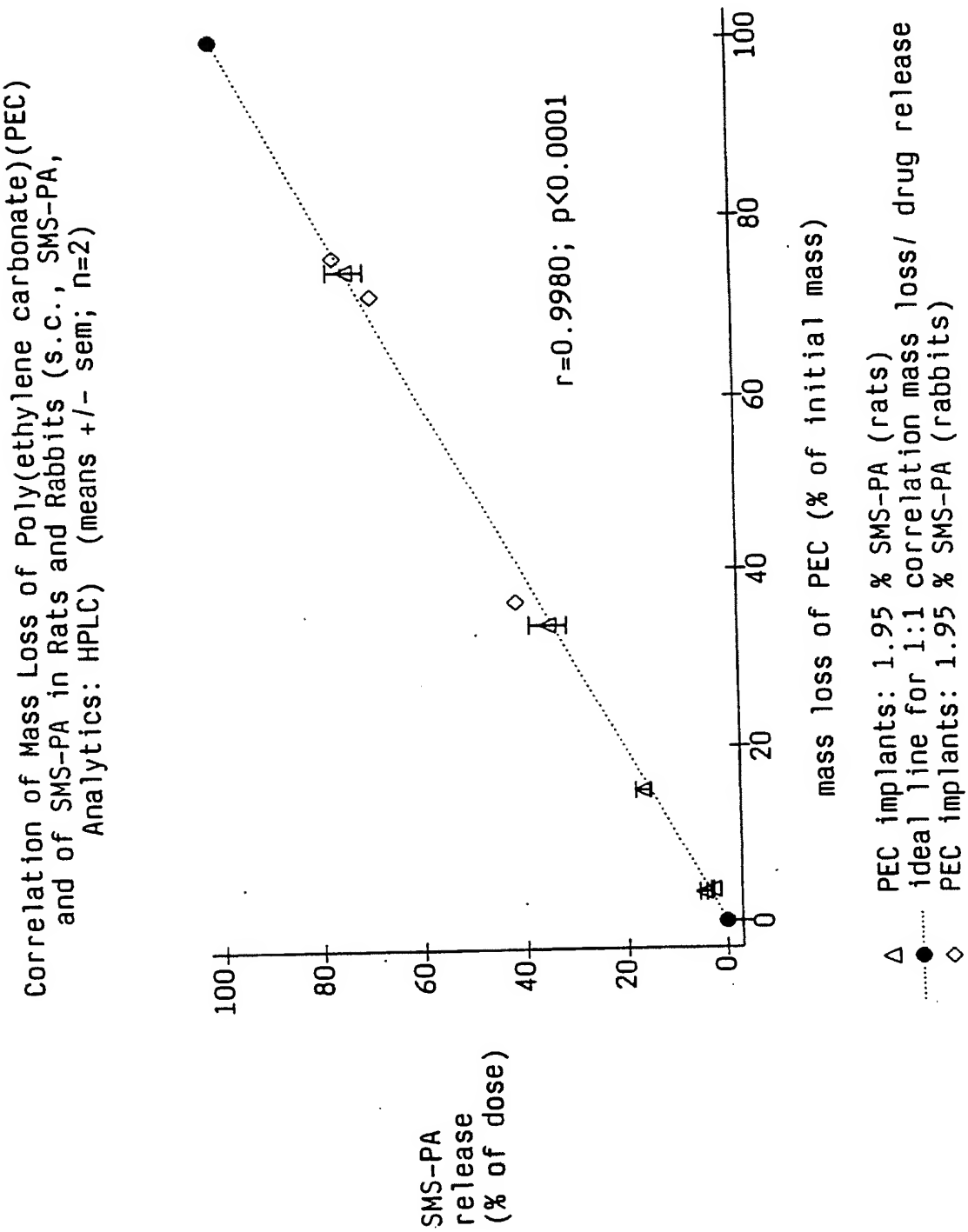

Plasma Levels of SMS-PA in Rats and in Rabbits
(means +/- sem; n=2-3)
SMS-PA plasma level (ng/ml)
0
5
10
15
0
2
4
6
8
10
12
14
16
18
20
22
implantation time (days)
--◆-- implants (rat)
--△-- implants (rabbit)
--▽-- microparticles (rabbit)

Fig. 13,


Correlation of Mass Loss of Poly(ethylene carbonate)(PEC)
and of SMS-PA in Rats and Rabbits (s.c., SMS-PA,
Analytics: HPLC) (means +/- sem; n=2)
r=0.9980; p<0.0001
SMS-PA
release
(% of dose)
mass loss of PEC (% of initial mass)
0
20
40
60
80
100
PEC implants: 1.95 % SMS-PA (rats)
ideal line for 1:1 correlation mass loss/ drug release
PEC implants: 1.95 % SMS-PA (rabbits)

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(57) Abstract

This invention provides pharmaceutical compositions comprising polymeric matrices, especially those comprising IL-6 as an active ingredient. Specific novel poly(ethylenecarbonate) polymers are also provided for more general use as matrix materials in sustained release compositions containing pharmacologically active compounds, as are methods of using of IL-6 for treatment of conditions mediated by IL-1 and/or TNFα, e.g., certain autoimmune and inflammatory conditions, as well as septic shock.

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 94/02833

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C08G64/02 C08G64/34 C08G64/18 A61K38/20 A61K9/16
A61K9/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C08G A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,3 953 383 (SHOHEI INOUE ET AL) 27 April 1976 see claims 1-7; example 2 ---	1-25
X	US,A,3 585 168 (SHOHEI INOUE) 15 June 1971 see column 2, line 65 - column 3, line 10; claims 1-4; example 13 ---	1-25
X	US,A,4 379 138 (PITT ET AL) 5 April 1983 see column 2, line 60 - line 6; claims 1,12; example 3 ---	26,27, 29,32-41
X	US,A,4 999 417 (DOMB) 21 March 1991 see claims 1-9; example 14 --- -/--	26,27, 29,32-41

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
22 December 1994	10. 04. 95
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer DECOCKER, L

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INTERNATIONAL SEARCH REPORT

Inter mal Application No
PCT/EP 94/02833

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
L	EP,A,0 535 937 (TAKEDA CHEMICAL INDUSTRIES, LTD) 7 April 1993 Cited in relation to non-unity see page 4, line 6; claim 1	
L	WO,A,93 11793 (SCHERING CORPORATION) 24 June 1993 Cited in relation to non-unity see claim 1	
L	DATABASE WPI Week 9206, Derwent Publications Ltd., London, GB; AN 92-045972 & JP,A,3 291 236 (TOSOH CORP) 20 December 1991 Cited in relation to non-unity see abstract	
A	CHEMICAL AND PHARMACEUTICAL BULLETIN, vol.31, no.4, 1983, TOKYO JP pages 1400 - 1403 TAKEO KAWAGUCHI ET AL 'Examination of biodegradability of poly(ethylene carbonate) and poly(propylene carbonate) in the peritoneal cavity in rats' cited in the application see the whole document	1

2

INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/EP 94/02833

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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because they relate to subject matter not required to be searched by this Authority, namely:

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because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see annex

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2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-42

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1. Claims 1-42: A specified biodegradable polymer, a process for its production, and a pharmaceutical composition comprising a pharmacologically active compound in a polymer showing non-hydrolytic surface erosion;
2. Claims 43-48: IL-6 in a polymeric matrix (as far as not comprised in claims 1-42)
3. Claims 49-54: The use of IL-6 in the manufacture of a medicament for certain therapeutic applications (as fas as not comprised in claims 1-48).

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/EP 94/02833

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-3953383	27-04-76	JP-C-	793935	30-10-75
		JP-A-	49031592	22-03-74
		JP-B-	50007039	20-03-75
		JP-C-	1039416	31-03-81
		JP-A-	49031591	22-03-74
		JP-B-	55029093	01-08-80
		DE-A-	2336854	14-02-74
		US-A-	3900424	19-08-75
US-A-3585168	15-06-71	NONE		
US-A-4379138	05-04-83	NONE		
US-A-4999417	12-03-91	EP-A-	0483429	06-05-92
EP-A-0535937	07-04-93	CA-A-	2079509	02-04-93
		JP-A-	5194200	03-08-93
WO-A-9311793	24-06-93	AU-B-	3244693	19-07-93